

## UTILITY

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860098.421C1

PATENT APPLICATION  
TRANSMITTAL

First Inventor or Application Identifier

Melanie Cobb

Title

TAO PROTEIN KINASE POLYPEPTIDES AND METHODS  
OF USE THEREFORE

Express Mail Label No.

EL487806495US

(Only for nonprovisional applications under 37 CFR § 1.53(b))

## APPLICATION ELEMENTS

See MPEP chapter 600 concerning utility patent application contents.

## ADDRESS TO:

Box Patent Application  
Assistant Commissioner for Patents  
Washington, D.C. 202311. ☐ General Authorization Form & Fee Transmittal  
(Submit an original and a duplicate for fee processing)2. ☒ Specification [Total Pages] **44**  
(preferred arrangement set forth below)

- Descriptive Title of the Invention
- Cross References to Related Applications
- Statement Regarding Fed sponsored R & D
- Reference to Microfiche Appendix
- Background of the Invention

- Brief Summary of the Invention
- Brief Description of the Drawings (if filed)
- Detailed Description
- Claim(s)
- Abstract of the Disclosure

3. ☒ Drawing(s) (35 USC 113) [Total Sheets] **20**4. Oath or Declaration [Total Pages] **20**

- a. ☐ Newly executed (original or copy)
- b. ☐ Copy from a prior application (37 CFR 1.63(d))  
(for continuation/divisional with Box 17 completed)
- i. ☐ **DELETION OF INVENTOR(S)**  
Signed statement attached deleting  
inventor(s) named in the prior application,  
see 37 CFR 1.63(d)(2) and 1.33(b)

Incorporation By Reference (useable if box 4b is  
checked) The entire disclosure of the prior application,  
from which a copy of the oath or declaration is supplied  
under Box 4b, is considered to be part of the disclosure of  
the accompanying application and is hereby incorporated  
by reference therein.

6. ☐ Microfiche Computer Program (Appendix)7. Nucleotide and Amino Acid Sequence Submission  
(if applicable, all necessary)

- a. ☒ Computer-Readable Copy
- b. ☒ Paper Copy (identical to computer copy)
- c. ☒ Statement verifying identity of above

## ACCOMPANYING APPLICATION PARTS

8. ☐ Assignment Papers (cover sheet & document(s))
9. ☐ 37 CFR 3.73(b) Statement ☐ Power of Attorney  
(when there is an assignee)
10. ☐ English Translation Document (if applicable)
11. ☐ Information Disclosure Statement (IDS)/PTO-1449 ☐ Copies of IDS Citations
12. ☐ Preliminary Amendment
13. ☒ Return Receipt Postcard
14. ☐ Small Entity Statement(s) ☐ Statement filed in prior application,  
Status still proper and desired
15. ☐ Certified Copy of Priority Document(s)  
(if foreign priority is claimed)
16. ☒ Other: Certificate of Express Mail

17. If a CONTINUING APPLICATION, check appropriate box and supply the requisite information below and in a preliminary amendment

☐ Continuation ☐ Divisional ☒ Continuation-In-Part (CIP) of prior Application No.: **09/060,410 filed 04/14/98**

Prior application information: Examiner \_\_\_\_\_ Group / Art Unit \_\_\_\_\_

☐ Claims the benefit of Provisional Application No. \_\_\_\_\_

## CORRESPONDENCE ADDRESS



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PATENT TRADEMARK OFFICE

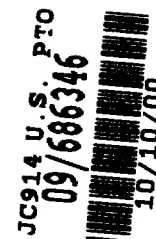
William T. Christiansen  
Seed Intellectual Property Law Group PLLC  
701 Fifth Avenue, Suite 6300  
Seattle, Washington 98104-7092  
Phone: (206) 622-4900  
Fax: (206) 682-6031

Respectfully submitted,

TYPED or PRINTED NAME William T. ChristiansenSIGNATURE William T. ChristiansenREGISTRATION NO. 44,614Date 10/10/00

PATENT  
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Melanie Cobb, Michele Hutchison, Zhu Chen and Kevin Berman  
Filed : October 10, 2000  
For : TAO PROTEIN KINASE POLYPEPTIDES AND METHODS  
OF USE THEREFORE



Docket No. : 860098.421C1  
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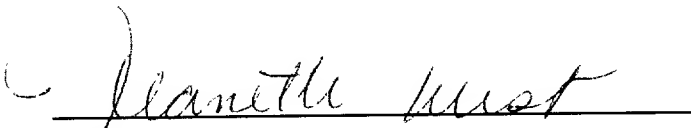
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Respectfully submitted,  
STMicronics, Inc.

  
Jeanette West

Enclosures:

Postcard  
Diskette  
Form PTO/SB/05  
Specification Claims, Abstract (44 pages)  
Declaration  
Sequence Listing (29 pages)  
20 Sheets of Drawings (Figures 1-20)

## STATEMENT OF GOVERNMENT INTEREST

### CROSS-REFERENCE TO PRIOR APPLICATION

TECHNICAL FIELD

The present invention relates generally to compositions and methods for modulating the activity of the MAP/ERK kinase MEK3 and/or other MEK family members. The invention is more particularly related to polypeptide variants of TAO proteins that have an enhanced ability to stimulate phosphorylation and activation of MEK substrates, such as MEK3. The invention is further related to the use of such proteins, for example, to activate a stress-responsive MAP kinase pathway in an organism and to identify antibodies and other agents that inhibit or activate signal transduction via such a pathway.

MAP kinase pathways are conserved signal transduction pathways that activate transcription factors, translation factors and other target molecules in response to a variety of extracellular signals. Each pathway contains a MAP kinase module, consisting of a MAP kinase or ERK, a MAP/ERK kinase (MEK), and a MEK kinase (MEKK). In higher eukaryotes, activation of MAP kinase pathways has been correlated with cellular events such as proliferation, oncogenesis, development and differentiation. Accordingly, the ability to regulate signal transduction via these pathways could lead to the development of treatments and preventive therapies for human diseases associated with MAP kinase pathways, such as inflammatory diseases, autoimmune diseases and cancer.

Several MAP kinase pathways have been found in *S. cerevisiae* (Hunter and Plowman, *Trends in Biochem. Sci.* 22:18-22, 1997), and parallel mammalian pathways have

been identified based upon sequences of mammalian ERKs and yeast MAP kinases, KSS1 and FUS3 (Boulton et al., *Science* 249:64-67, 1990; Courchesne et al., *Cell* 58: 1107-1119, 1989; Elion et al., *Cell* 60:649-664, 1990). The best delineated yeast MAP kinase pathway, activated by mating pheromones, is controlled by a receptor-G protein system, includes a Cdc42 small G protein, and requires at least three protein kinases, Ste20p (Leberer et al., *EMBO J.* 11:4815-4828, 1992; Ramer et al., *Proc. Natl. Acad. Sci. USA* 90:452-456, 1993), Ste11p (Rhodes et al., *Genes Dev.* 4:1862-1874, 1990), and Ste7p (Teague et al., *Proc. Natl. Acad. Sci. USA* 83:7371-7375, 1986), upstream of the MAP kinase Fus3p (Elion et al., *Cell* 60:649-664, 1990).

Ste20p was isolated from *S. cerevisiae* as a gene whose product functions downstream of the  $\beta\gamma$  subunits of a heterotrimeric G protein but upstream of enzymes in the MAP kinase module (MEKK, MEK, ERK) of the pheromone response pathway (Leberer et al., *EMBO J.* 11:4815-4828, 1992; Ramer et al., *Proc. Natl. Acad. Sci. USA* 90:452-456, 1993). Ste11p, the MEKK, may be one of the Ste20p substrates (Wu et al., *J. Biol. Chem.* 270:15984-15992, 1990); thus, Ste20p-like enzymes may activate MEKKs in mammalian MAP kinase pathways. Ste20p, like its best studied mammalian counterparts, the p21-activated protein kinases (PAKs), is thought to be regulated by binding to Cdc42 through a conserved Cdc42/Rac interactive binding region, or CRIB domain (Burbelo et al., *J. Biol. Chem.* 270:29071-29074, 1995).

Mammalian relatives of Ste20p are diverse and include the PAK subfamily (PAK1,2,3) and the mixed lineage kinase (MLK) subfamily, including the dual leucine zipper kinase (DLK), germinal center kinase (GCK), and the Nck-interacting kinase, NIK. In the past year, newly identified Ste20p-related kinases include members of the MLK subfamily, SOK-1, Krs-1 and -2, and MUK. MUK was isolated in a screen for MEKK isoforms, but in fact shows more identity to MLK. In transfected cells several of these enzymes, as first shown with GCK, increase the activity of the stress-responsive kinases, particularly SAPK/JNK. In the case of NIK and GCK, they may work by binding to MEKK (Su et al., *EMBO J.* 16:1279-1290, 1997). However, several of these Ste20p-related enzymes also have MEKK activity. For example, DLK phosphorylates and potentially activates MEKs that lie in the stress-responsive cascades.

Further characterization of members of these pathways, and the identification of additional members, is critical for understanding the signal transduction pathways involved and for developing methods for activating or inactivating MEKs and MAP kinase pathways *in vivo*. Accordingly, there is a need in the art for improved methods for modulating the activity of members of MAP kinase pathways, and for treating diseases associated with such pathways. The present invention fulfills these needs and further provides other related advantages.

#### SUMMARY OF THE INVENTION

Briefly stated, the present invention provides compositions and methods for modulating the activity of MAP/ERK kinases such as MEK3, and stress-responsive MAP kinase pathways. Within certain aspects, the present invention provides polypeptide variants of TAO proteins. Within one such aspect, polypeptide variants of TAO1 are provided, comprising an amino acid sequence that is at least 80% identical to residues 15-285 of SEQ ID NO:2, with the proviso that the variant does not comprise more than 500 consecutive amino acids of SEQ ID NO:2. Certain such variants comprise an amino acid sequence that is at least 90% identical to residues 15-285 of SEQ ID NO:2. Within certain embodiments, the variant comprises residues 1-416, 1-320 and/or 15-285 of SEQ ID NO:2.

Within other aspects, the present invention provides polypeptide variants of TAO2, comprising an amino acid sequence that is at least 80% identical to residues 15-285 of SEQ ID NO:4, with the proviso that the variant does not comprise more than 500 consecutive amino acids of SEQ ID NO:4. Certain such variants comprise an amino acid sequence that is at least 90% identical to residues 15-285 of SEQ ID NO:4. Within certain embodiments, the variant comprises residues 1-416, 1-320 and/or 15-285 of SEQ ID NO:4.

The present invention further provides, within other aspects, polypeptide variants of ceTAO, comprising an amino acid sequence that is at least 80% identical to residues 47-323 of SEQ ID NO:28, with the proviso that the variant does not comprise more than 500 consecutive amino acids of SEQ ID NO:28. Certain such variants comprise an amino acid sequence that is at least 90% identical to residues 47-323 of SEQ ID NO:28. Within certain embodiments, the variant comprises residues 1-454, 1-358 and/or 47-323 of SEQ ID NO:28.

Within further aspects, the present invention provides isolated polynucleotides encoding a polypeptide variant as described above. Certain such polynucleotides encode comprise at least 800 consecutive nucleotides any one of SEQ ID NOs:1, 3 or 27. Recombinant expression vectors comprising such a polynucleotide, as well as host cells transformed or transfected with such expression vectors are further provided.

Pharmaceutical compositions are also provided, within other aspects, comprising: (a) a polypeptide variant or polynucleotide as described above; and (b) a physiologically acceptable carrier.

The present invention further provides methods for phosphorylating a MEK polypeptide, comprising contacting a MEK polypeptide with a polypeptide variant as described above, wherein the MEK polypeptide comprises MEK3, MEK4 or MEK6 or a variant thereof, and thereby phosphorylating the MEK polypeptide.

Within further aspects, methods are provided for activating a member of a stress-responsive MAP kinase pathway in an organism, comprising administering to an organism a polypeptide variant as described above, and thereby activating a member of a stress-responsive MAP kinase pathway.

Within further aspects, methods are provided for screening for an agent that modulates signal transduction via a stress-responsive MAP kinase pathway, comprising: (a) contacting a candidate agent with a variant as described above; and (b) subsequently measuring the ability of the variant to modulate the activity of a MEK3 polypeptide, and thereby evaluating the ability of the compound to modulate signal transduction via a stress-responsive MAP kinase pathway.

These and other aspects of the present invention will become apparent upon reference to the following detailed description and attached drawings. All references disclosed herein are hereby incorporated by reference in their entirety as if each was incorporated individually.

## BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 presents the nucleotide and predicted amino acid sequence of a representative TAO1 kinase (SEQ ID NOs: 1 and 2).

Figure 2 presents a comparison of the catalytic domains of TAO1 (residues 1-273 of SEQ ID NO:2), TAO2 (residues 1-273 of SEQ ID NO:4), STE20 (SEQ ID NO:17) and the *C. elegans* homolog (ceTAO) (SEQ ID NO:18). The catalytic domains were aligned by eye and the conserved amino acids bolded. The domains are indicated with roman numerals.

Figures 3A and 3B are Northern blots, showing TAO1 (Figure 3A) and TAO2 (Figure 3B) expression in various tissues. Various rat poly-A<sup>+</sup> RNAs were probed, as indicated. Equal loading of RNA was verified by hybridizing the blot to an actin probe (not shown).

Figures 4A and 4B are Northern blots in which RNAs made from various human brain and spinal cord sections were hybridized to a TAO1-specific probe. Shown below each blot is the result of its hybridization to an actin probe. The lanes are as follows: 1, amygdala, 2, caudate nucleus, 3, corpus callosum, 4, hippocampus, 5, whole brain, 6, substantia nigra, 7, subthalamic nucleus, 8, thalamus, 9, cerebellum, 10, cerebral cortex, 11, medulla, 12, spinal cord, 13, occipital lobe, 14, frontal lobe, 15, temporal lobe, 16, putamen.

Figures 5A-5C are immunoblots. In Figure 5A, human embryonic kidney 293 cells were transiently transfected with either vector or pCMV5TAO1(HA)<sub>3</sub>, and 24 hours later lysates were immunoblotted with a monoclonal antibody directed against the HA epitope. TAO1 is indicated by the arrow. In Figure 5B, the TAO1 proteins purified from Sf9 cells were immunoblotted with an antibody directed against the MRGS(H)<sub>6</sub> epitope. In Figure 5C, 50ng of (His)<sub>6</sub>TAO1 was immunoblotted with polyclonal antisera P820 directed against a TAO1 peptide. An equal amount was blotted with the preimmune serum for P820.

Figure 6 is an autoradiogram showing the results of a representative *in vitro* linked kinase assay to estimate MEK activation by TAO1. Either 50 ng (lanes 1 and 3) or 250 ng (lanes 2 and 4) of (His)<sub>6</sub>TAO1(1-416) was incubated with 50 ng of (His)<sub>6</sub>MEK3 for one hour at 30° in the presence of Mg/ATP, after which a portion of the each reaction was added to a second reaction containing (His)<sub>6</sub>p38. After a one hour incubation, the reactions were subjected to SDS-PAGE and autoradiography.

Figure 7 is an autoradiogram showing the results of a representative *in vitro* linked kinase assay to estimate MEK activation by TAO1. Only the second part of the linked assay is shown. The assay was identical to that described in Figure 6, except that GSTMEK4

was substituted for MEK3, and both (His)<sub>6</sub>p38 and GSTSAPK $\beta$  were used as MEK4 substrates.

Figure 8 is an autoradiogram showing the results of a representative *in vitro* linked kinase assay to estimate MEK activation by TAO1. The assay was as described in Figures 6 and 7, but was performed with GSTMEK6 and (His)<sub>6</sub>p38 as the MEK6 substrate.

Figure 9 is a histogram comparing the fold activations of MEKs 1 through 6 by (His)<sub>6</sub>TAO1(1-416).

Figure 10 is an autoradiogram illustrating TAO1 activation of MEK3 *in vivo*. Human embryonic kidney 293 cells were transiently transfected with either vector alone, or pCMV5TAO1(HA)<sub>3</sub> and pCMV5mycMEK3, alone and in combination. Immunoprecipitates made with a monoclonal antibody directed against the myc epitope were subjected to *in vitro* kinase assays with (His)<sub>6</sub>p38 as substrate. Myc-tagged MEK3 expression detected with a polyclonal anti-MEK3 antisera is shown below. In several separate experiments, MEK3 activity in the immunoprecipitates was increased 3 to 4 fold when coexpressed with TAO1.

Figure 11 is an autoradiogram illustrating the copurification of TAO1 and endogenous MEK3 from Sf9 cells. Either 100 $\mu$ g of Sf9 whole cell lysate, or 1 $\mu$ g each of the recombinant TAO1 proteins purified from Sf9 cells was Western blotted with polyclonal antisera directed against MEK3 (top panel) or MEK4 (lower panel). An identical Western blot performed with an antisera against MEK6 did not detect MEK6 protein in either the Sf9 lysate or the TAO1 preparations.

Figure 12 presents an alignment of a human retina cDNA EST (sjbct; SEQ ID NO:5) with nts. 2341-2754 of the rat TAO1 kinase sequence (query) provided in Figure 1 (SEQ ID NO:1).

Figure 13 presents an alignment of a human retina cDNA EST (sjbct; SEQ ID NO:6) with nts. 964-651 of the rat TAO1 kinase sequence (query) provided in Figure 1 (SEQ ID NO:1).

Figure 14 presents an alignment of a human retina cDNA EST (sjbct; SEQ ID NO:7) with nts. 2792-2423 of the rat TAO1 kinase sequence (query) provided in Figure 1 (SEQ ID NO:1).

Figure 15A presents an alignment of a human retina cDNA EST (sjbct; SEQ ID NO:8) with nts. 2248-2437 of the rat TAO1 kinase sequence (query) provided in Figure 1



(SEQ ID NO:1). Figure 15B presents an alignment of a human retina cDNA EST (sbjct; SEQ ID NO:9) with nts. 2437-2501 of the rat TAO1 kinase sequence (query) provided in Figure 1 (SEQ ID NO:1).

Figure 16 presents an alignment of a human retina cDNA EST (sbjct; SEQ ID NO:10) with nts. 2087-2305 of the rat TAO1 kinase sequence (query) provided in Figure 1 (SEQ ID NO:1).

Figure 17A presents an alignment of a human retina cDNA EST (sbjct; SEQ ID NO:11) with nts. 3228-3312 of the rat TAO1 kinase sequence (query) provided in Figure 1 (SEQ ID NO:1). Figure 17B presents an alignment of a human retina cDNA EST (sbjct; SEQ ID NO:12) with nts. 3200-3245 of the rat TAO1 kinase sequence (query) provided in Figure 1 (SEQ ID NO:1).

Figure 18 presents an alignment of a human retina cDNA EST (sbjct; SEQ ID NO:13) with nts. 739-854 of the rat TAO1 kinase sequence (query) provided in Figure 1 (SEQ ID NO:1).

Figure 19A presents an alignment of a human retina cDNA EST (sbjct; SEQ ID NO:14) with nts. 526-643 of the rat TAO1 kinase sequence (query) provided in Figure 1 (SEQ ID NO:1). Figure 19B presents an alignment of a human retina cDNA EST (sbjct; SEQ ID NO:15) with nts. 187-296 of the rat TAO1 kinase sequence (query) provided in Figure 1 (SEQ ID NO:1).

Figure 20 presents an alignment of a human retina cDNA EST (sbjct; SEQ ID NO:16) with nts. 866-733 of the rat TAO1 kinase sequence (query) provided in Figure 1 (SEQ ID NO:1).

#### DETAILED DESCRIPTION OF THE INVENTION

As noted above, the present invention is generally directed to compounds and methods for modulating (*i.e.*, stimulating or inhibiting) the activity of MAP/ERK family members such as the MAP/ERK kinase MEK3. Compounds that activate such MEKs generally stimulate MEK phosphorylation. Such compounds include Ste20p homologs referred to herein as TAO proteins (*i.e.*, TAO1 (SEQ ID NO:2), TAO2 (SEQ ID NO:4), ceTAO (SEQ ID NO:28), as well as polypeptide variants of such proteins that retain the ability to stimulate MEK3 phosphorylation at a level that is not substantially lower than the

level stimulated by the native protein). Alternatively, a compound that activates MEK3 may comprise a polynucleotide that encodes a TAO polypeptide. Within other embodiments, compositions that stimulate MEK3 phosphorylation (thereby activating MEK3) may also, or alternatively, include one or more agents that stimulate TAO polypeptide expression or kinase activity. Such agents include, but are not limited to, stress-inducing agents (*e.g.*, DNA-damaging agents). Additional such agents may be identified by combining a test compound with a TAO polypeptide *in vitro* and evaluating the effect of the test compound on the kinase activity of the polypeptide using, for example, a representative assay described herein.

Preferred TAO polypeptides are those that comprise a C-terminal portion and have an activity (*i.e.*, the ability to stimulate MEK3 phosphorylation) that is comparable to, or enhanced relative to, a native TAO protein. Such polypeptides generally comprise at least the majority of the catalytic domain of a TAO protein (or a variant that is at least 80% identical the TAO protein catalytic domain), but do not comprise more than 500 consecutive amino acids of a TAO protein. For TAO1 or TAO2, a preferred polypeptide variant comprises residues 15-285; For ceTAO a preferred polypeptide variant comprises residues 47-323. It has been found, within the context of the present invention, that such truncated polypeptides may have substantially higher activity than the native TAO protein (*i.e.*, at least two fold higher, preferably at least 10 fold higher).

Compositions that inhibit the activity of MEKs generally inhibit MEK phosphorylation. Such compositions may include one or more agents that inhibit or block TAO polypeptide activity, such as an antibody that inhibits the kinase activity of a TAO polypeptide, a competing peptide that represents the substrate binding domain of a TAO protein or a phosphorylation motif of the MEK3 substrate, an antisense polynucleotide or ribozyme that interferes with transcription and/or translation of a TAO polypeptide, a molecule that inactivates a TAO polypeptide by binding to the polypeptide, a molecule that binds to the TAO substrate and prevents phosphorylation by a TAO polypeptide or a molecule that prevents transfer of phosphoryl groups from the kinase to the substrate. Agents that inhibit TAO polypeptide kinase activity may be identified by combining a test compound with a TAO polypeptide *in vitro* and evaluating the activity of the TAO polypeptide using a TAO kinase assay.

### TAO POLYNUCLEOTIDES

Any polynucleotide that encodes a TAO polypeptide, or a portion or variant thereof as described herein, is encompassed by the present invention. Such polynucleotides may be single-stranded (coding or antisense) or double-stranded, and may be DNA (genomic, cDNA or synthetic) or RNA molecules. Additional coding or non-coding sequences may, but need not, be present within a polynucleotide of the present invention, and a TAO polynucleotide may, but need not, be linked to other molecules and/or support materials. Preferred polynucleotides are those that encode a polypeptide having enhanced activity, relative to a native TAO protein.

Native TAO DNA sequences, or portions thereof, may be isolated using any of a variety of hybridization or amplification techniques, which are well known to those of ordinary skill in the art. Within such techniques, probes or primers may be designed based on the TAO sequences provided herein, and may be purchased or synthesized. Libraries from any suitable tissue (*e.g.*, brain) may be screened. An amplified portion or partial cDNA molecule may then be used to isolate a full length gene from a genomic DNA library or from a cDNA library, using well known techniques. Alternatively, a full length gene can be constructed from multiple PCR fragments.

Nucleic acid sequences corresponding to the native rat TAO polypeptides TAO1 and TAO2 are provided in SEQ ID NO:1 and SEQ ID NO:3, respectively; and the encoded amino acid sequences are provided in SEQ ID NOs:2 and 4, respectively. The predicted TAO1 open reading frame encodes a polypeptide of 1001 amino acids with a calculated molecular mass of 134 kD. TAO1 comprises an amino-terminal catalytic domain and an extensive carboxy-terminal region that has several distinguishing features, such as a possible nucleotide binding site and acidic stretch just carboxy-terminal to the catalytic domain, as well as two serine-rich regions. The TAO1 catalytic domain extends 263 amino acids from amino acid 25 to 288 with all 11 of the typical protein kinase subdomains conserved. There are two glutamate residues between TAO1 subdomains II and IV; the second glutamate at amino acid 76 contained in the sequence KEVK is most likely to represent subdomain III (Hanks et al., *Science* 241:42-52, 1988). The features of the TAO1 catalytic domain are most similar to the serine/threonine family of protein kinases;

subdomain VIb with the sequence HRDIKAGN (SEQ ID NO:26) suggests that TAO1 is likely to be a serine/threonine protein kinase. TAO2 has a similar arrangement of an amino-terminal kinase domain and a long carboxy-terminus, but differs in that it contains an acidic insert of 17 glutamate residues carboxy-terminal to the catalytic domain, and lacks the putative nucleotide binding site of TAO1.

As noted above, preferred polypeptide variants of TAO1 and TAO2 comprise an amino acid sequence that is at least 80% identical, and more preferably at least 90% identical, to residues 15-285 of a native TAO protein. Certain polypeptide variants comprise amino acids 1-320 or 1-416 of TAO1 or TAO2. Preferred polynucleotides encode such truncated variants, preferably variants with enhanced activity. For example, such TAO polynucleotides may comprise at least 800 consecutive nucleotides of a native sequence encoding TAO1 or TAO2.

ceTAO is the *Caenorhabditis elegans* TAO protein (Accession Number U32275; SEQ ID NO:28). Preferred variants of ceTAO comprise an amino acid sequence that is at least 80% identical, and more preferably at least 90% identical, to residues 47-323 of a native ceTAO. Certain such variants comprise amino acids 1-358 or 1-454 of ceTAO. Preferred polynucleotides encode such truncated variants, and particularly preferred TAO polynucleotides comprise at least 800 consecutive nucleotides of a native sequence encoding TAO1 or TAO2. Particularly preferred polynucleotides encode variants with enhanced activity.

The polynucleotides specifically recited herein, as well as full length polynucleotides comprising such sequences, other portions of full length polynucleotides, and sequences complementary to all or a portion of such full length molecules, are specifically encompassed by the present invention. In addition, TAO homologs from other species are specifically contemplated, and may generally be prepared as described herein for the rat homologs. In particular, within the context of the present invention, EST database sequences derived from retinal mRNAs have been identified that correspond to the human counterpart for TAO1. The sequences of these ESTs are provided in SEQ ID NOs:5-16. It will be readily apparent to those of ordinary skill in the art that a full length, native, human TAO1 polynucleotide may be identified based on such sequences, using for example, standard hybridization or amplification techniques. Such full length TAO1 sequences are

Polynucleotide variants of the recited sequences may differ from a native TAO polynucleotide in one or more substitutions, deletions, insertions and/or modifications. Certain variants encode a polypeptide that retains the ability to stimulate MEK3 phosphorylation at a level that is not substantially lower than the level stimulated by the native protein. The effect on the properties of the encoded polypeptide may generally be assessed as described herein. Preferred variants contain nucleotide substitutions, deletions, insertions and/or modifications at no more than 20%, preferably at no more than 10%, of the nucleotide positions. Certain variants are substantially homologous to a native gene, or a portion or complement thereof. Such polynucleotide variants are capable of hybridizing under moderately stringent conditions to a naturally occurring DNA sequence encoding a TAO protein (or a complementary sequence). Suitable moderately stringent conditions include prewashing in a solution of 5 X SSC, 0.5% SDS, 1.0 mM EDTA (pH 8.0); hybridizing at 50° C-65°C, 5 X SSC, overnight; followed by washing twice at 65°C for 20 minutes with each of 2X, 0.5X and 0.2X SSC containing 0.1% SDS). Such hybridizing DNA sequences are also within the scope of this invention.

It will be appreciated by those of ordinary skill in the art that, as a result of the degeneracy of the genetic code, there are many nucleotide sequences that encode a polypeptide as described herein. Some of these polynucleotides bear minimal homology to the nucleotide sequence of any native gene. Nonetheless, polynucleotides that vary due to differences in codon usage are specifically contemplated by the present invention.

As noted above, the present invention further provides antisense polynucleotides and portions of any of the above sequences. Such polynucleotides may generally be prepared by any method known in the art, including synthesis by, for example, solid phase phosphoramidite chemical synthesis. Alternatively, RNA molecules may be generated by *in vitro* or *in vivo* transcription of DNA sequences that are incorporated into a vector downstream of a suitable RNA polymerase promoter (such as T3, T7 or SP6). Certain portions of a TAO polynucleotide may be used to prepare an encoded polypeptide, as described herein. In addition, or alternatively, a portion may function as a probe (*e.g.*, to detect TAO expression in a sample), and may be labeled by a variety of reporter groups, such

as radionuclides, fluorescent dyes and enzymes. Such portions are preferably at least 10 nucleotides in length, and more preferably at least 20 nucleotides in length. Within certain preferred embodiments, a portion for use as a probe comprises a sequence that is unique to a TAO gene. A portion of a sequence complementary to a coding sequence (*i.e.*, an antisense polynucleotide) may also be used as a probe or to modulate gene expression. DNA constructs that can be transcribed into antisense RNA may also be introduced into cells or tissues to facilitate the production of antisense RNA.

Any polynucleotide may be further modified to increase stability *in vivo*. Possible modifications include, but are not limited to, the addition of flanking sequences at the 5' and/or 3' ends; the use of phosphorothioate or 2' O-methyl rather than phosphodiesterase linkages in the backbone; and/or the inclusion of nontraditional bases such as inosine, queosine and wybutosine, as well as acetyl- methyl-, thio- and other modified forms of adenine, cytidine, guanine, thymine and uridine.

Nucleotide sequences as described herein may be joined to a variety of other nucleotide sequences using established recombinant DNA techniques. For example, a polynucleotide may be cloned into any of a variety of cloning vectors, including plasmids, phagemids, lambda phage derivatives and cosmids. Vectors of particular interest include expression vectors, replication vectors, probe generation vectors and sequencing vectors. In general, a vector will contain an origin of replication functional in at least one organism, convenient restriction endonuclease sites and one or more selectable markers. Additional initial, terminal and/or intervening DNA sequences that, for example, facilitate construction of readily expressed vectors may also be present. Suitable vectors may be obtained commercially or assembled from the sequences described by methods well-known in the art. Other elements that may be present in a vector will depend upon the desired use, and will be apparent to those of ordinary skill in the art.

Vectors as described herein may generally be transfected into a suitable host cell, such as a mammalian cell, by methods well-known in the art. Such methods include calcium phosphate precipitation, electroporation and microinjection.

Polypeptides within the scope of the present invention comprise at least a portion of a TAO protein (*e.g.*, TAO1, TAO2 or ceTAO) or variant thereof, where the portion is immunologically and/or biologically active. Preferred variants retain the ability to stimulate MEK3 phosphorylation at a level that is not substantially lower than the level stimulated by the native protein. More preferably, a variant has enhanced ability to stimulate MEK3 phosphorylation (*e.g.*, at least two fold, five fold or ten fold), relative to the native protein. A polypeptide may further comprise additional sequences, which may or may not be derived from a native TAO protein. Such sequences may (but need not) possess immunogenic or antigenic properties and/or a biological activity.

A polypeptide "variant," as used herein, is a polypeptide that differs from a native protein in substitutions, insertions, deletions and/or amino acid modifications, such that the immunogenic and/or biological properties of the native protein are not substantially diminished. A variant preferably retains at least 80% sequence identity to a native sequence, more preferably at least 90% identity, and even more preferably at least 95% identity. Within certain preferred embodiments, such variants contain alterations at no more than 20% of the amino acid residues in the native polypeptide, such that the ability of the variant to stimulate MEK3 phosphorylation is enhanced. Guidance in determining which and how many amino acid residues may be substituted, inserted, deleted and/or modified without diminishing immunological and/or biological activity may be found using any of a variety of methods and computer programs known in the art. Properties of a variant may generally be evaluated by assaying the reactivity of the variant with, for example, antibodies as described herein and/or evaluating a biological property characteristic of the native protein.

A polypeptide is "immunologically active," within the context of the present invention if it is recognized (*i.e.*, specifically bound) by a B-cell and/or T-cell surface antigen receptor. Immunological activity may generally be assessed using well known techniques, such as those summarized in Paul, *Fundamental Immunology*, 3rd ed., 243-247 (Raven Press, 1993) and references cited therein. Such techniques include screening polypeptides derived from the native polypeptide for the ability to react with antigen-specific antisera and/or T-cell lines or clones, which may be prepared using well known techniques. An immunologically active portion of a TAO protein reacts with such antisera and/or T-cells at a level that is not substantially lower than the reactivity of the full length polypeptide (*e.g.*, in an ELISA and/or

T-cell reactivity assay). Such screens may generally be performed using methods well known to those of ordinary skill in the art, such as those described in Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988. B-cell and T-cell epitopes may also be predicted via computer analysis.

Similarly, a polypeptide is "biologically active" if the ability to phosphorylate MEK3 and/or other MEKs is not substantially diminished within a representative *in vitro* assay as described in Example 3. As used herein, the term "not substantially diminished" means retaining an activity that is at least 90% of the activity of a native TAO protein. Preferably, the ability of the polypeptide to phosphorylate MEK3 is enhanced at least two fold, preferably at least five fold and more preferably at least ten fold. Appropriate assays designed to evaluate such activity may be designed based on existing assays known in the art, and on the representative assays provided herein.

Preferred variants contain conservative substitutions. A "conservative substitution" is one in which an amino acid is substituted for another amino acid that has similar properties, such that one skilled in the art of peptide chemistry would expect the secondary structure and hydrophathic nature of the polypeptide to be substantially unchanged. Amino acid substitutions may generally be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity and/or the amphipathic nature of the residues. For example, negatively charged amino acids include aspartic acid and glutamic acid; positively charged amino acids include lysine and arginine; and amino acids with uncharged polar head groups having similar hydrophilicity values include leucine, isoleucine and valine; glycine and alanine; asparagine and glutamine; and serine, threonine, phenylalanine and tyrosine. Other groups of amino acids that may represent conservative changes include: (1) ala, pro, gly, glu, asp, gln, asn, ser, thr; (2) cys, ser, tyr, thr; (3) val, ile, leu, met, ala, phe; (4) lys, arg, his; and (5) phe, tyr, trp, his. A variant may also, or alternatively, contain nonconservative changes.

In general, modifications may be more readily made in non-critical regions, which are regions of the native sequence that do not substantially change the properties of the TAO protein. Non-critical regions may be identified by modifying the TAO sequence in a particular region and assaying the activity of the resulting variant in a kinase assay, using MEK3, MEK4, MEK6 or another MEK family member as a substrate, as described herein.



Variants of TAO proteins may include constitutively active proteins. In general, activation of a TAO protein *in vivo* requires stimulation by a stimulus such as a stress-inducing agent. Constitutively active variants display the ability to stimulate MEK phosphorylation in the absence of such stimulation. Such variants may be identified using the representative *in vivo* assays for TAO kinase activity described herein.

Variants within the scope of this invention also include polypeptides in which the primary amino acid structure of a native protein is modified by forming covalent or aggregative conjugates with other polypeptides or chemical moieties such as glycosyl groups, lipids, phosphate, acetyl groups and the like. Covalent derivatives may be prepared, for example, by linking particular functional groups to amino acid side chains or at the N- or C-termini.

The present invention also includes polypeptides with or without associated native-pattern glycosylation. Polypeptides expressed in yeast or mammalian expression systems may be similar to or slightly different in molecular weight and glycosylation pattern



In general, any of a variety of expression vectors known to those of ordinary skill in the art may be employed to express recombinant polypeptides of this invention. Expression may be achieved in any appropriate host cell that has been transformed or transfected with an expression vector containing a DNA sequence that encodes a recombinant polypeptide. Suitable host cells include prokaryotes, yeast, baculovirus-infected insect cells and animal cells. Following expression, supernatants from host/vector systems which secrete recombinant protein or polypeptide into culture media may be first concentrated using a commercially available filter. Following concentration, the concentrate may be applied to a suitable purification matrix such as an affinity matrix or an ion exchange resin. One or more reverse phase HPLC steps can be employed to further purify a recombinant polypeptide.

Portions and other variants having fewer than about 100 amino acids, and generally fewer than about 50 amino acids, may also be generated by synthetic means, using techniques well known to those of ordinary skill in the art. For example, such polypeptides may be synthesized using any of the commercially available solid-phase techniques, such as the Merrifield solid-phase synthesis method, where amino acids are sequentially added to a growing amino acid chain. *See Merrifield, J. Am. Chem. Soc. 85:2149-2146, 1963.* Various modified solid phase techniques are also available (e.g., the method of Roberge et al., *Science* 269:202-204, 1995). Equipment for automated synthesis of polypeptides is commercially available from suppliers such as Applied BioSystems, Inc. (Foster City, CA), and may be operated according to the manufacturer's instructions.

In general, polypeptides and polynucleotides as described herein are isolated. An "isolated" polypeptide or polynucleotide is one that is removed from its original environment. For example, a naturally-occurring protein is isolated if it is separated from some or all of the coexisting materials in the natural system. Preferably, polypeptides provided herein are isolated to a purity of at least 80% by weight, more preferably to a purity of at least 95% by weight, and most preferably to a purity of at least 99% by weight. In general, such purification may be achieved using, for example, the standard techniques of ammonium sulfate fractionation, SDS-PAGE electrophoresis, and affinity chromatography. A polynucleotide is considered to be isolated if, for example, it is cloned into a vector that is not a part of the natural environment.

The present invention further provides antibodies, and antigen-binding fragments thereof, that specifically bind to a TAO polypeptide. As used herein, an antibody, or antigen-binding fragment, is said to "specifically bind" to a TAO polypeptide if it reacts at a detectable level (within, for example, an ELISA) with a TAO polypeptide, and does not react detectably with unrelated proteins. Antibodies may be polyclonal or monoclonal. Preferred antibodies are those antibodies that inhibit or block TAO activity *in vivo* and within a kinase assay as described herein. Other preferred antibodies (which may be used, for example, in immunokinase assays) are those that immunoprecipitate active TAO1 and/or TAO2.

Antibodies may be prepared by any of a variety of techniques known to those of ordinary skill in the art (*see, e.g.,* Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988). In one such technique, an immunogen comprising the polypeptide is initially injected into a suitable animal (*e.g.,* mice, rats, rabbits, sheep and goats), preferably according to a predetermined schedule incorporating one or more booster immunizations, and the animals are bled periodically. Polyclonal antibodies specific for the polypeptide may then be purified from such antisera by, for example, affinity chromatography using the polypeptide coupled to a suitable solid support.

Monoclonal antibodies specific for a TAO polypeptide may be prepared, for example, using the technique of Kohler and Milstein, *Eur. J. Immunol.* 6:511-519, 1976, and improvements thereto. Briefly, these methods involve the preparation of immortal cell lines capable of producing antibodies having the desired specificity (*i.e.*, reactivity with the polypeptide of interest). Such cell lines may be produced, for example, from spleen cells obtained from an animal immunized as described above. The spleen cells are then immortalized by, for example, fusion with a myeloma cell fusion partner, preferably one that is syngeneic with the immunized animal. For example, the spleen cells and myeloma cells may be combined with a nonionic detergent for a few minutes and then plated at low density on a selective medium that supports the growth of hybrid cells, but not myeloma cells. A preferred selection technique uses HAT (hypoxanthine, aminopterin, thymidine) selection. After a sufficient time, usually about 1 to 2 weeks, colonies of hybrids are observed. Single

Monoclonal antibodies may be isolated from the supernatants of growing hybridoma colonies. In addition, various techniques may be employed to enhance the yield, such as injection of the hybridoma cell line into the peritoneal cavity of a suitable vertebrate host, such as a mouse. Monoclonal antibodies may then be harvested from the ascites fluid or the blood. Contaminants may be removed from the antibodies by conventional techniques, such as chromatography, gel filtration, precipitation, and extraction.

## METHODS AND KITS FOR DETECTING TAO POLYPEPTIDES AND TAO KINASE ACTIVITY

There are a variety of assay formats known to those of ordinary skill in the art for using an antibody to detect a polypeptide in a sample. See, e.g., Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988. For example, the antibody may be immobilized on a solid support such that it can bind to and remove the polypeptide from the sample. The bound polypeptide may then be detected using a second antibody that binds to the antibody/peptide complex and contains a detectable reporter group.

For detecting an active TAO protein in a sample, an immunokinase assay may be employed. Briefly, polyclonal or monoclonal antibodies may be raised against a unique sequence of a TAO protein (such as amino acid residues 296-315, 403-418, 545-563 or 829-848) using standard techniques. A sample to be tested, such as a cellular extract, is incubated with the anti-TAO antibodies to immunoprecipitate a TAO protein, and the immunoprecipitated material is then incubated with a substrate (*e.g.*, MEK3) under suitable conditions for substrate phosphorylation. The level of substrate phosphorylation may generally be determined using any of a variety of assays, as described herein.

TAO kinase assays, for use in evaluating the polypeptide variants and other agents discussed herein, include any assays that evaluate a compound's ability to phosphorylate MEK3 or other MEKs, thereby rendering the MEK active (*i.e.*, capable of phosphorylating *in vivo* substrates such as p38). MEKs such as MEK3 for use in such methods may be endogenous proteins or variants thereof, may be purified or recombinant, and may be prepared using any of a variety of techniques that will be apparent to those of ordinary skill in the art. For example, cDNA encoding MEK3 may be cloned by PCR amplification from a suitable human cDNA library, using polymerase chain reaction (PCR) and methods well known to those of ordinary skill in the art. MEK3 may be cloned using primers based on the published sequence (Derijard et al., *Science* 267:682-685, 1995). MEK3 cDNA may then be cloned into a bacterial expression vector and the protein produced in bacteria, such as *E. coli*, using standard techniques. The bacterial expression vector may, but need not, include DNA encoding an epitope such as glutathione-S transferase protein (GST) such that the recombinant protein contains the epitope at the N- or C-terminus.

A TAO kinase assay may generally be performed as described herein. Briefly, a TAO polypeptide may be incubated with MEK3 and [ $\gamma$ - $^{32}$ P]ATP in a suitable buffer (such as 50 mM HEPES pH 8, 10 mM MgCl<sub>2</sub>, 1 mM DTT, 100  $\mu$ M ATP) for 60 minutes at 30°C. In general, approximately 50 ng to 1  $\mu$ g of the polypeptide and 50 ng recombinant MEK3, with 2-7 cpm/fmol [ $\gamma$ - $^{32}$ P]ATP, is sufficient. Proteins may then be separated by SDS-PAGE on 10% gels and subjected to autoradiography. Incorporation of [ $^{32}$ P]phosphate into MEK3 may be quantitated using techniques well known to those of ordinary skill in the art, such as with a phosphorimager. To evaluate the substrate specificity of polypeptide variants, a kinase assay may generally be performed as described above except that other MEK substrates (*i.e.*, MEK1, 2, 4 or 6) are substituted for the MEK3.

To determine whether MEK3 phosphorylation results in activation, a coupled *in vitro* kinase assay may be performed using a substrate for MEK3, such as p38, with or without an epitope tag. p38 for use in such an assay may be prepared as described in Han et al., *J. Biol. Chem.* 271:2886-2891, 1996. Briefly, following phosphorylation of MEK3 as described above, the MEK3 (*e.g.*, 0.1-10 ng) may be incubated with p38 (*e.g.*, 10  $\mu$ g/ml) and [ $\gamma$ - $^{32}$ P]ATP in a kinase buffer as described herein. It should be noted that alternative buffers may be used and that buffer composition can vary without significantly altering kinase activity. Reactions may be separated by SDS-PAGE, visualized by autoradiography and quantitated using any of a variety of known techniques. Activated MEK3 will be capable of phosphorylating p38 at a level that is at least 5% above background using such an assay.

The present invention further provides kits for detecting TAO polypeptides and TAO kinase activity. Such kits may be designed for detecting the level of a TAO polypeptide or polynucleotide, or may detect phosphorylation of MEK3 in a direct kinase assay or a coupled kinase assay, in which the level of phosphorylation and/or the kinase activity of MEK3 may be determined. TAO polypeptides and TAO kinase activity may be detected in any of a variety of samples, such as eukaryotic cells, bacteria, viruses, extracts prepared from such organisms and fluids found within living organisms. In general, the kits of the present invention comprise one or more containers enclosing elements, such as reagents or buffers, to be used in the assay.

A kit for detecting the level of TAO polypeptide or polynucleotide typically contains a reagent that binds to TAO1 and/or TAO2 protein, DNA or RNA. To detect

A kit for detecting TAO kinase activity based on measuring the phosphorylation of MEK3 generally comprises MEK3 in combination with a suitable buffer. A kit for detecting TAO kinase activity based on detecting MEK3 activity generally comprises MEK3 in combination with a suitable MEK3 substrate, such as p38. Optionally, the kit may additionally comprise a suitable buffer and/or material for purification of MEK3 after activation and before combination with substrate. Such kits may be employed in direct or coupled kinase assays, which may be performed as described above.

The present invention further provides methods for identifying antibodies and other compounds that bind to and/or modulate the activity of a TAO polypeptide. To evaluate the effect of a candidate modulating agent on TAO polypeptide activity, a kinase assay may be performed as described above, except that the candidate modulating agent is added to the incubation mixture. Briefly, the reaction components, which include the composition to be tested and the TAO polypeptide or a polynucleotide encoding the kinase, are incubated under conditions sufficient to allow the components to interact. Subsequently, the effect of composition on kinase activity or on the level of a polynucleotide encoding the kinase is measured. The observed effect on the kinase may be either inhibitory or stimulatory. The increase or decrease in kinase activity can be measured by, for example, adding a radioactive compound such as  $^{32}\text{P}$ -ATP to the mixture of components, and observing radioactive incorporation into MEK3 or other suitable substrate for a TAO

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Within such assays, the candidate agent may be preincubated with a TAO polypeptide before addition of ATP and substrate. Alternatively, the substrate may be preincubated with the candidate agent before the addition of kinase. Further variations include adding the candidate agent to a mixture of TAO polypeptide and ATP before the addition of substrate, or to a mixture of substrate and ATP before the addition of TAO polypeptide. Any of these assays can further be modified by removing the candidate agent after the initial preincubation step. In general, a suitable amount of antibody or other candidate agent for use in such an assay ranges from about 0.1  $\mu\text{M}$  to about 10  $\mu\text{M}$ . The effect of the agent on TAO kinase activity may then be evaluated by quantitating the incorporation of [ $^{32}\text{P}$ ]phosphate into MEK3, as described above, and comparing the level of incorporation with that achieved using the TAO polypeptide without the addition of the candidate agent.

Alternatively, a whole cell system may employ only the transactivation domain of ATF2 fused to a suitable DNA binding domain, such as GHF-1 or GAL4. The reporter system may then comprise the GH-luciferase or GAL4-luciferase plasmid. Candidate TAO protein modulating agents may then be added to the system to evaluate their effect on ATF2-specific gene activation.

In other aspects of the subject invention, methods for using the above polypeptides to phosphorylate and activate MEK3, peptide derivatives thereof or other MEK family members are provided. MEK substrate for use in such methods may be prepared as described above. In one embodiment, MEK3 may be phosphorylated *in vitro* by incubation with a TAO polypeptide and ATP in a suitable buffer as described above. In general, the amounts of the reaction components may range from about 0.1  $\mu$ g to about 10  $\mu$ g of TAO polypeptide, from about 0.1  $\mu$ g to about 10  $\mu$ g of recombinant MEK3, and from about 100 nM to about 1 mM (preferably about 100 pmol - 30 nmol) of ATP. Phosphorylated proteins may then be purified by binding to GSH-Sepharose and washing. The extent of MEK3 phosphorylation may generally be monitored by adding [ $\gamma$ - $^{32}$ P]ATP to a test aliquot, and evaluating the level of MEK3 phosphorylation as described above. The activity of the phosphorylated MEK3 may be evaluated using a coupled *in vitro* kinase assay, as described above.

Once activated *in vitro*, MEK3 may be used, for example, to identify agents that inhibit the kinase activity of MEK3. Such inhibitory agents, which may be antibodies or drugs, may be identified using the coupled assay described above. Briefly, a candidate agent may be included in the mixture of MEK3 and p38, with or without pre-incubation with one or more components of the mixture, as described above. In general, a suitable amount of antibody or other agent for use in such an assay ranges from about 0.1  $\mu$ M to about 10  $\mu$ M. The effect of the agent on MEK3 kinase activity may then be evaluated by quantitating the incorporation of [ $^{32}$ P]phosphate into p38, as described above, and comparing the level of incorporation with that achieved using activated MEK3 without the addition of a candidate agent.

Within other aspects, TAO polypeptides may be used to identify one or more native upstream kinases (*i.e.*, kinases that phosphorylate and activate TAO1 and/or TAO2 *in vivo*, or other signaling molecules that regulate TAO activity). TAO polypeptides may be used in a yeast two-hybrid system to identify interacting proteins. Alternatively, an expression library may be screened to identify cDNAs that encode proteins which phosphorylate a TAO polypeptide. Other methods for identifying such upstream kinases may also be employed, and will be apparent to those of ordinary skill in the art.

For administration to a patient, one or more polypeptides, polynucleotides, antibodies and/or modulating agents are generally formulated as a pharmaceutical composition, which may be a sterile aqueous or non-aqueous solution, suspension or emulsion, and which additionally comprises a physiologically acceptable carrier (*i.e.*, a non-toxic material that does not interfere with the activity of the active ingredient). Any suitable carrier known to those of ordinary skill in the art may be employed in a pharmaceutical composition. Representative carriers include physiological saline solutions, gelatin, water, alcohols, natural or synthetic oils, saccharide solutions, glycols, injectable organic esters such as ethyl oleate or a combination of such materials. Such compositions may also comprise buffers (*e.g.*, neutral buffered saline or phosphate buffered saline), carbohydrates (*e.g.*, glucose, mannose, sucrose or dextrans), mannitol, proteins, polypeptides or amino acids such as glycine, antioxidants, antimicrobial compounds, chelating agents such as EDTA or glutathione, adjuvants (*e.g.*, aluminum hydroxide), inert gases and/or preservatives. Compositions of the present invention may also be formulated as a lyophilizate. Pharmaceutical compositions may also contain other compounds, which may be biologically active or inactive.

The compositions described herein may be administered as part of a sustained release formulation (*i.e.*, a formulation such as a capsule that effects a slow release of compound following administration). Such formulations may generally be prepared using well known technology and administered by, for example, oral, rectal or subcutaneous implantation, or by implantation at the desired target site. Sustained-release formulations may contain a polypeptide, polynucleotide or modulating agent dispersed in a carrier matrix and/or contained within a reservoir surrounded by a rate controlling membrane. Carriers for use within such formulations are biocompatible, and may also be biodegradable; preferably the formulation provides a relatively constant level of release. The amount of active compound contained within a sustained release formulation depends upon the site of implantation, the rate and expected duration of release and the nature of the condition to be treated or prevented.

Certain pharmaceutical compositions contain DNA encoding a polypeptide, antibody fragment or other modulating agent as described above (such that a TAO

polypeptide, a variant thereof or a modulating agent is generated *in situ*) or an antisense polynucleotide. In such pharmaceutical compositions, the DNA may be present within any of a variety of delivery systems known to those of ordinary skill in the art, including nucleic acid, bacterial and viral expression systems, as well as colloidal dispersion systems, including liposomes. Appropriate nucleic acid expression systems contain the necessary DNA sequences for expression in the patient (such as a suitable promoter and terminating signal). The DNA may also be "naked," as described, for example, in Ulmer et al., *Science* 259:1745-1749, 1993.

Various viral vectors that can be used to introduce a nucleic acid sequence into the targeted patient's cells include, but are not limited to, vaccinia or other pox virus, herpes virus, retrovirus, or adenovirus. Techniques for incorporating DNA into such vectors are well known to those of ordinary skill in the art. Preferably, the retroviral vector is a derivative of a murine or avian retrovirus including, but not limited to, Moloney murine leukemia virus (MoMuLV), Harvey murine sarcoma virus (HaMuSV), murine mammary tumor virus (MuMTV), and Rous Sarcoma Virus (RSV). A retroviral vector may additionally transfer or incorporate a gene for a selectable marker (to aid in the identification or selection of transduced cells) and/or a gene that encodes the ligand for a receptor on a specific target cell (to render the vector target specific). For example, retroviral vectors can be made target specific by inserting a nucleotide sequence encoding a sugar, a glycolipid, or a protein. Targeting may also be accomplished using an antibody, by methods known to those of ordinary skill in the art.

Viral vectors are typically non-pathogenic (defective), replication competent viruses, which require assistance in order to produce infectious vector particles. This assistance can be provided, for example, by using helper cell lines that contain plasmids that encode all of the structural genes of the retrovirus under the control of regulatory sequences within the LTR, but that are missing a nucleotide sequence which enables the packaging mechanism to recognize an RNA transcript for encapsulation. Such helper cell lines include (but are not limited to) Ψ2, PA317 and PA12. A retroviral vector introduced into such cells can be packaged and vector virion produced. The vector virions produced by this method can then be used to infect a tissue cell line, such as NIH 3T3 cells, to produce large quantities of chimeric retroviral virions.

Another targeted delivery system for TAO polynucleotides is a colloidal dispersion system. Colloidal dispersion systems include macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. A preferred colloidal system for use as a delivery vehicle *in vitro* and *in vivo* is a liposome (*i.e.*, an artificial membrane vesicle). It has been shown that large unilamellar vesicles (LUV), which range in size from 0.2-4.0  $\mu\text{m}$  can encapsulate a substantial percentage of an aqueous buffer containing large macromolecules. RNA, DNA and intact virions can be encapsulated within the aqueous interior and be delivered to cells in a biologically active form (Fraley, et al., *Trends Biochem. Sci.* 6:77, 1981). In addition to mammalian cells, liposomes have been used for delivery of polynucleotides in plant, yeast and bacterial cells. In order for a liposome to be an efficient gene transfer vehicle, the following characteristics should be present: (1) encapsulation of the genes of interest at high efficiency while not compromising their biological activity; (2) preferential and substantial binding to a target cell in comparison to non-target cells; (3) delivery of the aqueous contents of the vesicle to the target cell cytoplasm at high efficiency; and (4) accurate and effective expression of genetic information (Mannino, et al., *Biotechniques* 6:882, 1988).

The targeting of liposomes can be classified based on anatomical and mechanistic factors. Anatomical classification is based on the level of selectivity, for example, organ-specific, cell-specific, and organelle-specific. Mechanistic targeting can be distinguished based upon whether it is passive or active. Passive targeting utilizes the natural tendency of liposomes to distribute to cells of the reticuloendothelial system (RES) in organs which contain sinusoidal capillaries. Active targeting, on the other hand, involves alteration of the liposome by coupling the liposome to a specific ligand such as a monoclonal antibody, sugar, glycolipid, or protein, or by changing the composition or size of the liposome in order to achieve targeting to organs and cell types other than the naturally occurring sites of localization.

Routes and frequency of administration, as well as polypeptide, modulating agent or nucleic acid doses, will vary from patient to patient. In general, the pharmaceutical compositions may be administered intravenously, intraperitoneally, intramuscularly, subcutaneously, intracavity or transdermally. Between 1 and 6 doses may be administered

daily. A suitable dose is an amount of polypeptide or DNA that is sufficient to show improvement in the symptoms of a patient afflicted with a disease associated with a stress-responsive MAP kinase pathway. Such improvement may be detected based on a determination of relevant cytokine levels (*e.g.*, IL-2, IL-8), by monitoring inflammatory responses (*e.g.*, edema, transplant rejection, hypersensitivity) or through an improvement in clinical symptoms associated with the disease. In general, the amount of polypeptide present in a dose, or produced *in situ* by DNA present in a dose, ranges from about 1  $\mu$ g to about 250  $\mu$ g per kg of host, typically from about 1  $\mu$ g to about 60  $\mu$ g. Suitable dose sizes will vary with the size of the patient, but will typically range from about 10 mL to about 500 mL for 10-60 kg animal.

#### THERAPEUTIC APPLICATIONS

The above polypeptides, polynucleotides and/or modulating agents may be used to phosphorylate (and thereby activate) MEK3, or to inhibit such phosphorylation, in a patient. As used herein, a "patient" may be any mammal, including a human, and may be afflicted with a disease associated with a stress-responsive MAP kinase pathway, or may be free of detectable disease. Accordingly, the treatment may be of an existing disease or may be prophylactic. Diseases associated with a stress-responsive MAP kinase pathway include any disorder which is etiologically linked to a TAO protein kinase activity, including immune-related diseases (*e.g.*, inflammatory diseases, autoimmune diseases, malignant cytokine production or endotoxic shock), cell growth-related diseases (*e.g.*, cancer, metabolic diseases, abnormal cell growth and proliferation or cell cycle abnormalities) and cell regeneration-related diseases (*e.g.*, cancer, degenerative diseases, trauma, environmental stress by heat, UV or chemicals or abnormalities in development and differentiation). Immunological-related cell proliferative diseases such as osteoarthritis, ischemia, reperfusion injury, trauma, certain cancers and viral disorders, and autoimmune diseases such as rheumatoid arthritis, diabetes, multiple sclerosis, psoriasis, inflammatory bowel disease, and other acute phase responses may also be treated.

Treatment includes administration of a composition or compound which modulates the kinase activity of TAO1 and/or TAO2. Such modulation includes the suppression of TAO expression and/or activity when it is over-expressed, or augmentation of

As noted above, antibodies, polynucleotides and other agents having a desired effect on TAO expression and/or activity may be administered to a patient (either prophylactically or for treatment of an existing disease) to modulate the activation of MEK3 *in vivo*. For example, an agent that decreases TAO activity *in vivo* may be administered to prevent or treat inflammation, autoimmune diseases, cancer or degenerative diseases. In particular, such agents may be used to prevent or treat insulin-resistant diabetes, metabolic disorders and neurodegenerative diseases. In general, for administration to a patient, an antibody or other agent is formulated as a pharmaceutical composition as described above. A suitable dose of such an agent is an amount sufficient to show benefit in the patient based on the criteria noted above.

The following Examples are offered by way of illustration and not by way of limitation.

## EXAMPLES

Example 1Cloning and Sequencing cDNA Encoding TAO1 and TAO2

This Example illustrates the cloning of cDNA molecules encoding the rat Ste20p-related protein kinases TAO1 and TAO2, and the identification of the human TAO1 homolog.

First-strand cDNA from adult rat brain was used as the template in the first round of PCR with degenerate oligonucleotide primers derived from the Ste20p sequence, 5'-GACGCTGGATCCAA(AG)AT(ACT)GGICA(AG)GGIGC-3' (SEQ ID NO:19) and 5'-GGIGTICC(AG)TTIGTIGCIAT-3' (SEQ ID NO:20). A portion of the product of this reaction was used as the template in a second round of PCR with nested primers, also derived from the Ste20p sequence, 5'-AA(AG)GA(AG)CAIATI(CA)TIAA(CT)GA(AG)AT-3' (SEQ ID NO:21) and 5'-GACGCTGAATTAC(CT)TCIGGIGCCATCCA-3' (SEQ ID NO:22). The resulting 420 base product was labeled with [ $\alpha$ - $^{32}$ P]dCTP by random-priming, and used to probe approximately  $1 \times 10^6$  plaques of an oligo(dT) and random-primed  $\lambda$ ZAP library generated from adult rat forebrain RNA. In excess of 100 positive clones were obtained; of those sequenced, all contained regions of overlap with the original PCR product. A full length TAO1 sequence was assembled from two overlapping cDNAs, using the SacI site at nucleotide 50 to insert a fragment of TAO1 cDNA including nucleotides 50 to 3003. The full length TAO1 sequence is shown in Figure 1 and SEQ ID NO:1.

The TAO1 open reading frame encodes 1001 amino acids, with a calculated molecular mass of 134kDa. The presumed initiator codon begins at base 121 and is preceded by an in-frame stop codon at base 106. The longest 5' UTR obtained was 600 nucleotides in length, and the longest 3' UTR was 1200 nucleotides. None of the clones analyzed contained a poly-A track.

As is the case with most protein kinases, TAO1 can be divided into regions based on amino acid sequence comparison to other protein kinases. TAO1 is composed of an amino-terminal catalytic domain and an extensive carboxy-terminal region that has several distinguishing features, such as a possible nucleotide binding site and acidic stretch just



The TAO1 catalytic domain extends 263 amino acids from amino acid 25 to 288 with all 11 of the typical protein kinase subdomains conserved. There are two glutamate residues between TAO1 subdomains II and IV; the second glutamate at amino acid 76 contained in the sequence KEVK is most likely to represent subdomain III (Hanks et al., *Science* 241:42-52, 1988). The features of the TAO1 catalytic domain are most similar to the serine/threonine family of protein kinases; subdomain VIb with the sequence HRDIKAGN suggests that TAO1 is likely to be a serine/threonine protein kinase.

In the process of screening the cDNA library for clones near the 5' end of TAO1, multiple clones representing a second closely related gene (TAO2) were identified. The TAO2 sequence is provided in SEQ ID NO:3, with the predicted amino acid sequence shown in SEQ ID NO:4 and Figure 2. TAO2 is highly related to TAO1, and has a similar arrangement of an amino-terminal kinase domain and a long carboxy-terminus, but differs in that it contains an acidic insert of 17 glutamate residues carboxy-terminal to the catalytic domain, and lacks the putative nucleotide binding site of TAO1.

Sequences from EST databases derived from retinal mRNAs revealed the human counterpart for TAO1. The EST sequences identified are provided in SEQ ID NOs:5-16, and the alignments of these sequences with the rat TAO1 sequence are provided in Figures 12-20.

The FASTA program was used to compare the percent amino acid identities of several protein kinase catalytic domains, and the results are presented in Table 1, below.

Table 1

	TAO1							
TAO2	90	TAO2						
ceTAO	65	61	ceTAO					
STE20d	40	39	37	STE20				
GCK	43	42	35	40	GCK			
MLK1	32	30	27	30	29	MLK1		
MST1	47	43	42	42	47	28	MST1	
MEKK1	34	33	27	30	30	30	29	

To assess the expression of TAO1 in transfected cells, full-length, HA-tagged TAO1 cDNA was transfected into human embryonic kidney 293 cells. A protein of approximately 140kDa could be detected by Western blotting with an antibody directed against the HA epitope (Figure 5A). The observed molecular mass of the protein is in good agreement with the mass predicted from the cDNA sequence.

### Example 2

#### *In vivo* Expression of TAO1 and TAO2

This Example illustrates the expression of TAO1 and TAO2 in a variety of adult rat and human tissues, as determined by Northern blot analysis.

Total RNA isolated from various adult male rat tissues was selected for poly-A+ RNA with oligo(dT)cellulose (Collaborative Biomedical Products) according to the manufacturers protocols, and 5µg of each RNA was subjected to Northern analysis. The PCR-generated 420 base fragment derived from the catalytic domain of TAO1 (described above) was labeled with [ $\alpha$ -<sup>32</sup>P]dCTP by random-priming and used to probe the Northern blot. Hybridization was at 42°C, followed by washing at 55°C in 0.2%SSC/0.1%SDS. Integrity of the mRNA was confirmed by hybridization to an actin probe. The TAO1 probe hybridized predominately to an mRNA species of approximately 12kb, and less strongly to another of approximately 10kb (Figure 3A). Of the rat tissues examined, brain clearly showed the strongest hybridization signal. On prolonged exposure, heart and lung revealed weak hybridization signals, while in skeletal muscle, liver, kidney, testis, epididymus, and spleen no signal was detected.

To assess the expression pattern of TAO2, the rat tissue Northern blot was stored until the hybridization signal for TAO1 was not seen on a two week exposure at -80°C. A fragment from the catalytic domain of TAO2 was labeled with [ $\alpha$ -<sup>32</sup>P]dCTP by random priming, and used to probe the Northern under the same hybridization and washing conditions described above for TAO1.

When the same rat tissue Northern blot was probed with a fragment of the catalytic domain of TAO2, the strongest hybridization signal was also seen in brain. The size of the transcript hybridizing to the TAO2 probe was smaller than that seen for TAO1, at 5kb (Figure 3B).

A probe from the non-catalytic carboxy-terminus of TAO1 (corresponding to nucleotides 1555 to 2632 of TAO1 (*see* Figure 1)) was used for all additional Northern analyses because it is less likely to hybridize to TAO2 mRNA. This probe from the carboxy-terminus of TAO1 was used to assess the expression pattern in sections of human brain (Clontech). Hybridizations were performed at 68°C in Clontech ExpressHyb buffer, and washed at 55°C as per the manufacturer's instructions.

The strongest hybridization signals were seen in amygdala, corpus callosum, hippocampus, and substantia nigra, and each of these was stronger than that seen in whole brain (Figure 4A). Weaker signals were seen in caudate nucleus, subthalamic nucleus and thalamus. A second human brain Northern hybridized to the same probe showed strong

hybridization signals in cerebellum, putamen and occipital, frontal and temporal lobes, but much weaker signals in cerebral cortex, medulla and spinal cord (Figure 4B).

### Example 3

#### Kinase Activity and Substrate Specificity of TAO1

This Example illustrates the kinase activity and substrate specificity of TAO1, in *in vitro* and *in vivo* assays.

To determine whether TAO1 is active as a protein kinase, two constructs were employed. pCMV5TAO1-HA<sub>3</sub> and pCMV5TAO1(1-416)-HA<sub>3</sub> were generated by cloning the cDNAs encoding these TAO1 polypeptides into the pCMV5 mammalian expression vector. Oligonucleotide primers were used with TAO1 cDNA as template to amplify a 1247 base pair DNA product encoding amino acids 1 to 416. This fragment contains all 11 of the kinase subdomains (with the initial methionine deleted). The resulting constructs were transfected into human embryonic kidney 293 cells, and the recombinant, tagged proteins immunoprecipitated with an antibody directed against the HA epitope.

*In vitro* kinase assays were generally performed as follows. Kinase assays contained: 50mM Hepes, pH 8, 10mM MgCl<sub>2</sub>, 1mM DTT, 100μM ATP, [γ-<sup>32</sup>P]ATP (at a final concentration of 2-7 cpm/fmol), and unless otherwise noted, reactions were incubated at 30°C for 60 minutes in a 30μl volume. Protein kinase substrates such as myelin basic protein were added at a final concentration of 0.5 mg/ml. Reactions were halted by the addition of 10μl 5X Laemmli buffer, followed by boiling, and 20μl were analyzed by SDS-PAGE and autoradiography. For linked kinase assays, 50-250ng of recombinant TAO1 protein was incubated with 50ng of each of the bacterially expressed MEK proteins in a 30μl reaction volume for 60 minutes at 30°C, and then 5μl of this reaction was added to a second reaction mix containing bacterially expressed (His)<sub>6</sub>p38 or GST-SAPKβ at a final concentration of 10μg/ml. Recombinant MEK proteins were kindly provided by Andrei Khokhlatchev and Megan Robinson, and may be prepared as described by Robinson et al., *J. Biol. Chem.* 271:29734-29739, 1996 and references cited therein. Within such assays, both TAO1(1-416) and full-length TAO1 were able to phosphorylate MBP in immune complex kinase reactions.

To quantitate the activity of more highly purified TAO1, TAO1(1-416), full-length TAO1 and full-length TAO1(D169A) were expressed with an amino-terminal hexa-histidine tag in Sf9 cells. TAO1(D169A) is a catalytically defective TAO1 mutant, which was created by changing aspartic acid 169 to an alanine (D169A) with PCR, and cloning the resulting construct into the pCMV5 mammalian expression vector. These constructs were prepared with either a single hemagglutinin (HA) epitope tag at the amino-terminus, a triple HA epitope tag at the carboxy-terminus, or a myc epitope tag at the amino-terminus.

The recombinant, hexa-histidine tagged TAO1, TAO1(1-416), and TAO1(D169A) were expressed in *Spodoptera frugiperda* (Sf9) cells. Cells were lysed by douncing in 50mM sodium phosphate, pH 8.5, 1mM DTT, 1mM PMSF, and 1mg/ml each leupeptin, pepstatin A, and aprotinin. After centrifugation at 30,000xg for 30 minutes, the supernatant was applied to a Ni<sup>2+</sup>-NTA agarose (Qiagen) column pre-equilibrated with the same buffer. The column was then washed with 50 column volumes of buffer, and eluted with a 20 ml gradient of 0 to 250mM imidazole, all in the above buffer. Fractions containing recombinant TAO1 proteins were detected in fractions by Western blotting with an antibody to the MRGS(H)<sub>6</sub> epitope (Qiagen), and appropriate fractions were pooled and dialyzed to remove the imidazole.

(His)<sub>6</sub>TAO1(1-416) expressed as a single 57kDa band (Figure 5B). Both the (His)<sub>6</sub>TAO1 and (His)<sub>6</sub>TAO1(D169A) recombinant proteins migrated as 140kDa bands, although the D169A mutant appears to be more subject to degradation. (His)<sub>6</sub>TAO1(1-416) phosphorylates MBP with a specific activity of 1  $\mu\text{molmin}^{-1}\text{mg}^{-1}$  in the presence of 1mM ATP. Full-length (His)<sub>6</sub>TAO1 exhibits MBP phosphorylating activity that is comparable to the 1-416 truncation mutant, while the activity of TAO1(D169A) is reduced to 90% of that of the wild-type protein. (His)<sub>6</sub>TAO1(1-416) was also able to phosphorylate  $\alpha$ -casein, histone I, and histone 7.

To determine whether TAO1 activates one or more of the known MEKs, (His)<sub>6</sub>TAO1(1-416) was incubated with bacterially produced MEK for one hour in the presence of Mg<sup>2+</sup> and [ $\gamma$ -<sup>32</sup>P]ATP. A portion of this reaction was then transferred to a similar reaction containing the appropriate bacterially expressed MEK substrate, (His)<sub>6</sub>ERK2K52R for MEK1 and MEK2, (His)<sub>6</sub>p38 for MEK3 and MEK6, and (His)<sub>6</sub>p38 and GST-SAPK $\beta$  for

(His)<sub>6</sub>TAO1(1-416) activated GST-MEK4 5-fold toward (His)<sub>6</sub>p38, and 150-fold towards GST-SAPKβ (Figure 7). The difference in fold activation seen for MEK4 towards the two substrates probably reflects the difference in basal kinase activity of MEK4 towards p38 and SAPKβ *in vitro*. TAO1 also increased the ability of GST-MEK6 to phosphorylate (His)<sub>6</sub>p38, by 5-fold (Figure 8). Recombinant GST-MEK5 was not phosphorylated by (His)<sub>6</sub>TAO1(1-416).

The degree of activation of each of the MEK proteins by (His)<sub>6</sub>TAO1(1-416) *in vitro* is comparable to that seen by a bacterially produced amino-terminal truncation of MEKK1 (Xu et al., *Proc. Natl. Acad. Sci. USA* 92:6808-6812, 1995; Robinson et al., *J. Biol. Chem.* 271:29734-29739, 1996). To distinguish the MEK-activating ability of TAO1 from that of MEKK, the ability of (His)<sub>6</sub>TAO1(1-416) to activate MEK1 and MEK2 was assessed. As shown in Figure 9, (His)<sub>6</sub>TAO1(1-416) was completely unable to increase the activity of MEK1 or MEK2 towards the substrate (His)<sub>6</sub>ERK2 under the same conditions that TAO1 activates MEK3, MEK4, and MEK6. Thus, while TAO1 displays MEKK-like activity in its ability to activate various MEKs, TAO1 is differentiated from MEKK by its inability to recognize MEK1 and MEK2. Figure 9 shows the fold activation of the various MEKs by TAO1.

To assess the ability of TAO1 to activate the various MEKs *in vivo*, full-length HA-tagged TAO1 was co-transfected into 293 cells with myc-tagged MEK3, or myc-tagged TAO1 was co-transfected with HA-tagged MEK4 or HA-tagged MEK6. The pCMV5myc-MEK3 construct was generated by inserting the MEK3 coding sequence

(provided by K.L. Guan, University of Michigan, which may be prepared as described by Robinson et al., *J. Biol. Chem.* 271:29734-29739, 1996) into the pCMV5Myc vector, such that the Myc epitope is at the amino-terminus of MEK3. The MEKs were then immunoprecipitated and added to immune complex kinase assays with the appropriate substrate and  $Mg^{2+}$ /ATP. In multiple experiments, myc-tagged MEK3 showed a 3-fold higher activity toward p38 when immunoprecipitated from 293 cells co-expressing TAO than from cells not transfected with TAO (Figure 10). In contrast, TAO was not able to increase the activity of immunoprecipitated HA-tagged MEK4 towards GST-SAPK $\beta$ , or that of HA-tagged MEK6 toward p38.

In transfected cells, TAO1 activates MEK3 3-fold, but neither MEK4 nor MEK6. The selectivity in transfected cells may arise from the ability of TAO1 to bind MEK3. The endogenous MEK3 from Sf9 cells copurifies with recombinant TAO1 expressed in the cells. These findings suggest that TAO1 may be an important regulator of the p38 pathway.

To determine which MEK3 residues are phosphorylated by TAO, an *in vitro* kinase reaction was performed with (His)<sub>6</sub>TAO1(1-416) and (His)<sub>6</sub>MEK3; the 57kDa band corresponding to TAO1 and the 30 kDa band corresponding to MEK3 were excised and treated as described. Phosphoproteins were separated by SDS-PAGE, transferred to Immobilon-P membrane (Millipore) electrophoretically, and visualized by autoradiography. Bands of interest were excised and hydrolyzed in 6M HCl for 60 minutes at 110°C. The hydrolysate was dried under vacuum, and resuspended in a 2.2% formic acid, 12% acetic acid solution at an activity of 2000cpm/ $\mu$ l. Then 1 $\mu$ l of each sample was mixed with 1 $\mu$ g each of the three phosphoamino acid standards, and spotted onto cellulose thin-layer chromatography plates. Electrophoresis was performed in 0.5% pyridine, 5% acetic acid at 1200 volts for 60 minutes. After air drying the plates, the standards were visualized with 0.25% ninhydrin in acetone. Autoradiography revealed only phosphoserine and phosphothreonine in both (His)<sub>6</sub>TAO1(1-416) and (His)<sub>6</sub>MEK3 (Figure 11).

Example 4  
Co-Purification of MEK3 and TAO1

This Example shows that TAO1 and MEK3 co-purify.

Although the ability of (His)<sub>6</sub>TAO1 to activate MEK3 was always reduced in comparison with that of (His)<sub>6</sub>TAO1(1-416), several assays showed that the ability of (His)<sub>6</sub>TAO1 to lead to an increase in the phosphorylation of p38 in the linked kinase assays was partly independent of the addition of MEK. (His)<sub>6</sub>TAO1(1-416) does not phosphorylate p38. Therefore, Western analyses were performed to determine if one or more MEKs might be present in the TAO1 preparations purified from Sf9 cells.

(His)<sub>6</sub>TAO1, (His)<sub>6</sub>TAO1(1-416), and (His)<sub>6</sub>TAO1(D169A) were subjected to Western analysis with antisera specific to MEK3, MEK4, and MEK6. Four different polyclonal antisera were raised to these three TAO1 peptides in rabbits. The peptide TKDAVRELDNLQYRKMKKLL (SEQ ID NO:23) corresponding to the amino acids 296 to 315 yielded antisera P820. The peptide KKELNSFLESQKREYKLRK (SEQ ID NO:24) of amino acids 545 to 563 yielded the antiserum R562. Finally, the peptide RELRELEQQRVSLRRALLEQK (SEQ ID NO:25) of amino acids 829 to 848 resulted in the antisera R564 and R565. These peptides were conjugated to *Limulus* hemocyanin (Boulton and Cobb, *Cell. Regul.* 2:357-371, 1991) and dialyzed into phosphate-buffered saline. A total of five boosts were performed, after which the rabbits were exsanguinated and the serum collected. The antisera were screened for reactivity by Western blotting of recombinant TAO1 expressed in Sf9 cells. Five antisera were found to consistently recognize the recombinant TAO1 protein in Western blots. Free peptide was able to block the specific recognition of TAO1 protein by the antisera. None of the five antisera detected the presence of TAO1 in lysates of 293, NIH3T3, NG-108, or COS cells.

For immunoblot analysis, either 50ng of recombinant TAO1 protein or 100 µg of cell lysate was subjected to SDS-PAGE, then transferred to nitrocellulose membranes. The membranes were blocked with 5% nonfat powdered milk in TBST (20mM Tris, pH 8, 500mM NaCl, 0.05% Tween 20) for one hour, then incubated with the polyclonal antisera at 1:500 dilution in TBST plus 0.25% milk for one hour. After three washes with TBST, the membranes were incubated with a 1:2500 dilution of horseradish peroxidase-conjugated



MEK3 was clearly seen in the (His)<sub>6</sub>TAO1 preparation, and to a lesser extent in the (His)<sub>6</sub>TAO1(D169A) preparation (Figure 11). MEK4 was detected in the Sf9 cell lysates, but not in the TAO1 preparations, while MEK6 was detected in neither.

### TAO Polypeptide Variants with Enhanced Activity

PBluescript-TAO2(1-320), containing the catalytic domain of TAO2 was generated by PCR. Wild-type TAO2 and TAO2(1-320) were cloned into pTSETB (Invitrogen) to incorporate a MRGSH<sub>6</sub> tag and were subsequently transferred to the baculovirus shuttle vector pVL1393. Recombinant viruses were selected, and recombinant protein was harvested, as described by Hutchison et al., *J. Biol. Chem.* 273:28625-28632, 1998.

Proteins were adsorbed to Ni<sup>2+</sup>-nitrilotriacetic acid agarose (Qiagen) and eluted with a gradient of 20-250 mM imidazole in 0.5 mM dithiothreitol (DTT) and 0.3 M NaCl. TAO2 was detected by Western blotting with an antibody to the MRGSH<sub>6</sub> epitope (Qiagen) and silver staining.

Activity was assessed using *in vitro* kinase assays as described above, using 0.5 mg/mL myelin basic protein (MBP) as the substrate. The truncated, recombinant TAO2 (1-320) phosphorylated MBP with a specific activity of 0.6  $\mu\text{mol min}^{-1} \text{mg}^{-1}$ . The full length protein had lower activity, about 10% of the truncated enzyme.

From the foregoing, it will be appreciated that, although specific embodiments of the invention have been described herein for the purpose of illustration, various modifications may be made without deviating from the spirit and scope of the invention.

Claims

1. A polypeptide variant of TAO1, comprising an amino acid sequence that is at least 80% identical to residues 15-285 of SEQ ID NO:2, with the proviso that the variant does not comprise more than 500 consecutive amino acids of SEQ ID NO:2.

2. A variant according to claim 1, wherein the amino acid sequence is at least 90% identical to residues 15-285 of SEQ ID NO:2.

3. A variant according to claim 1, wherein the variant comprises residues 1-416 of SEQ ID NO:2.

4. A variant according to claim 1, wherein the variant comprises residues 1-320 of SEQ ID NO:2.

5. A variant according to claim 1, wherein the variant comprises residues 15-285 of SEQ ID NO:2.

6. A polypeptide variant of TAO2, comprising an amino acid sequence that is at least 80% identical to residues 15-285 of SEQ ID NO:4, with the proviso that the variant does not comprise more than 500 consecutive amino acids of SEQ ID NO:4.

7. A variant according to claim 6, wherein the amino acid sequence is at least 90% identical to residues 15-285 of SEQ ID NO:4.

8. A variant according to claim 6, wherein the variant comprises residues 1-416 of SEQ ID NO:4.

9. A variant according to claim 6, wherein the variant comprises residues 1-320 of SEQ ID NO:4.

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20. A recombinant expression vector comprising a polynucleotide according to claim 16.

21. A host cell transformed or transfected with an expression vector according to claim 20.

22. A pharmaceutical composition, comprising:

- (a) a variant according to any one of claims 1-15; and
- (b) a physiologically acceptable carrier.

23. A pharmaceutical composition, comprising:

- (a) a polynucleotide according to claim 16; and
- (b) a physiologically acceptable carrier.

24. A method for phosphorylating a MEK polypeptide, comprising contacting a MEK polypeptide with a variant according to any one of claims 1, 6 or 10, wherein the MEK polypeptide comprises MEK3, MEK4 or MEK6 or a variant thereof, and thereby phosphorylating the MEK polypeptide.

25. A method for activating a member of a stress-responsive MAP kinase pathway in an organism, comprising administering to an organism a variant according to any one of claims 1, 6 or 10, and thereby activating a member of a stress-responsive MAP kinase pathway.

26. The method of claim 25 wherein the member of the stress-responsive MAP kinase pathway is MEK3.

27. A method for screening for an agent that modulates signal transduction via a stress-responsive MAP kinase pathway, comprising:

- (a) contacting a candidate agent with a variant according to any one of claims 1, 6 or 10; and



TAO PROTEIN KINASE POLYPEPTIDES AND METHODS OF USE THEREFORAbstract of the Disclosure

Compositions and methods are provided for potentiating the activity of the mitogen-activated protein kinase p38. In particular the mitogen-activated protein kinase kinase MEK6, and variants thereof that stimulate phosphorylation of p38 are provided. Such compounds may be used, for example, for therapy of diseases associated with the p38 cascade and to identify antibodies and other agents that inhibit or activate signal transduction via p38.

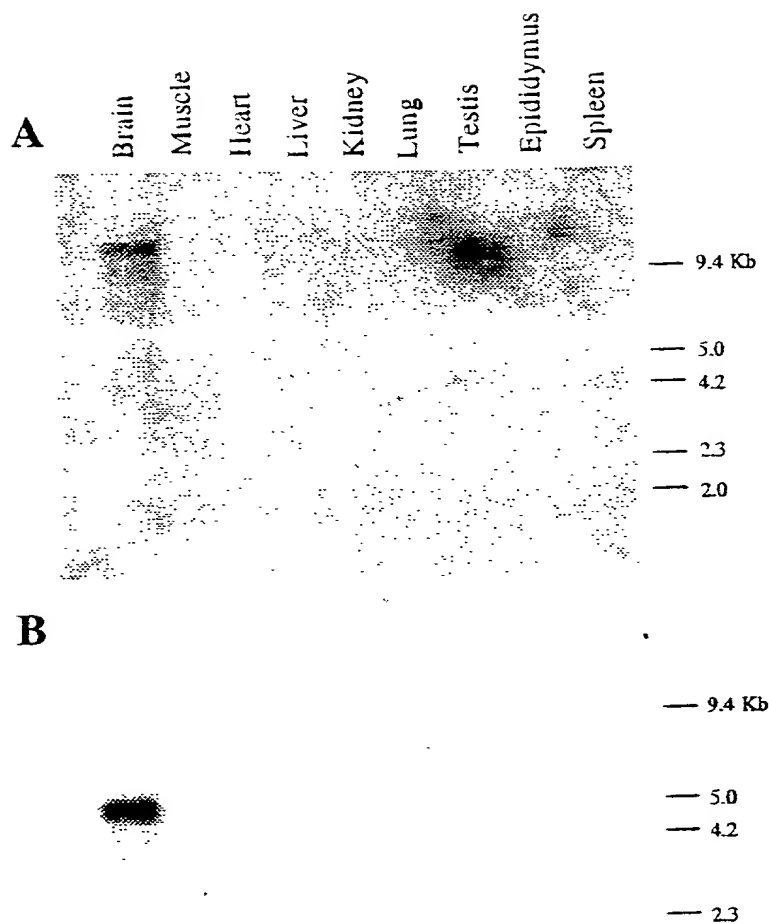
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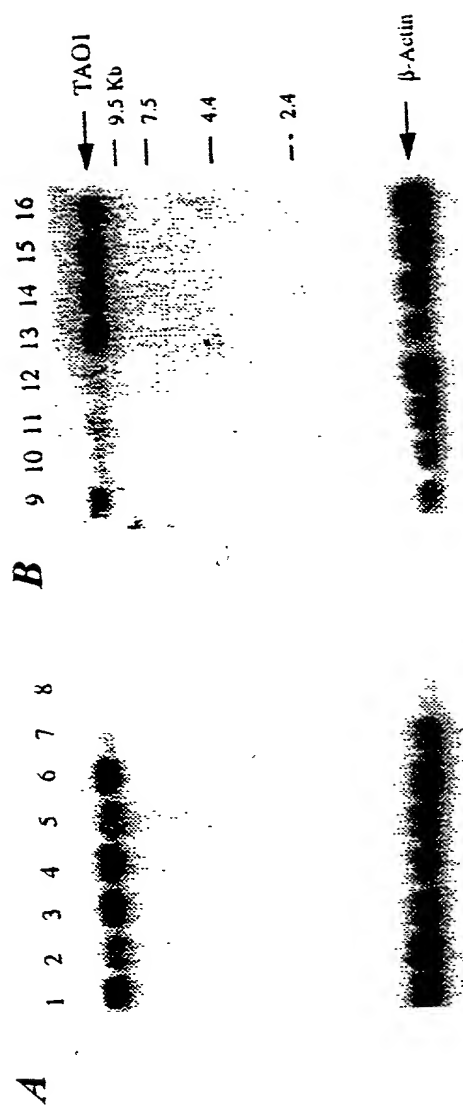
	I	II	III		
TA01	MPSTHRAGSLKDPETAELEFFKEDPEKLF	TDLREIGHOSFOAVYFAKQVNTNEVVAIKKMSYSGKQSTERNW	-QDI IREV	77	
TA02	MPAGGRAGSLKDPVVAELFFKDDPEKLF	SOLREIGHOSFOAVYFARDVRNSEVVAIKKMSYSGKQSNERNW	-QDI IREV	77	
CTAO	-MAPAVLQKIVIKIPSTAAALF	SNKDPEDLREIGHOSFOAVYFYDKRNEQVVAIKKMSYSGKQAVEKNW	-NDILREV	76	
STE20	HEERERKKQLYAKLNEICSDGDPSTRYANLVKIQQASOGVYTAI	EIGTHVSVAIKQANLE	-KQPKKEI IIRILLVMK	670	
	IV	V	VI		
TA01	KFLORIKHPNSLEYKGCYLREHTAWLVMEYCIOSASD	LLEVIHKKPLQEVETAAITHGALQGLAYLHSHHTM	IHRDIKAGN	156	
TA02	RFLQKLRHPNTIQYROCYLEHTAWLVMEYCIOSASE	FELEVIHKKPLQEVETAAVTHGALQGLAYLHSHHTM	IHRDVKAGN	156	
CTAO	SFLNTVNIPIHIVDYKACFLDITTCMLVMEYCIOSAA	DIVDLRKKGMREVEIAALCSQTLDALRYLHSLKR	IHRDIKAGN	155	
STE20	Q----	SKHPNIVNFI	DSYVLKGDWVIMETMEGOSLTDV	VTIHCILTEGQICAVCRETLSGLEFLHSGKGVIIHRDIKSDM	744
	VII	VIII	IX		
TA01	ILLTEPGQVKLADFGSAS----	MASPANSFVOTFPYVMAPEFVILAMDEGQYDGKVDVMBLQITCIELAERKPEPLFNNA	210		
TA02	ILLSEFGVLKIGDDFGSAS----	IMAPANSFVOTFPYVMAPEFVILAMDEGQYDGKVDVMBLQITCIELADRKPEPLFNNA	210		
CTAO	ILLSHDAIVKLADFGSAS----	LVDPAQTFFIOTPPFMAPEFVILAMDEGHYTDRAIDIMBLQITCIELAERRPEPLFSNA	229		
STE20	ILLSHEGDINKLTDFOFCAQINELNLKRTTMTVOTFPYVMAPEFVVS	SRKE---	XGPKVDIWSLQIMIMIEGEPEPYLNETP	819	
	X	XI			
TA01	MSALYHIAQNESPTLQSNWSDYFRN-----	FVDSCLQKIPQDRPTSE	273		
TA02	MSALYHIAQNESPALQSNHWSYFRN-----	FVDSCLQKIPQDRPTSE	273		
CTAO	MSALYHIAQNDPPTLSPIDTSEQPEHSELEFVQPIDKCLRKPAEERMSAE	279			
STE20	LRLALYLIAITNGTPKIKPENLSS---	SLKK--	7LDNCLCIVEPEDEKASAT	863	

FIG. 2

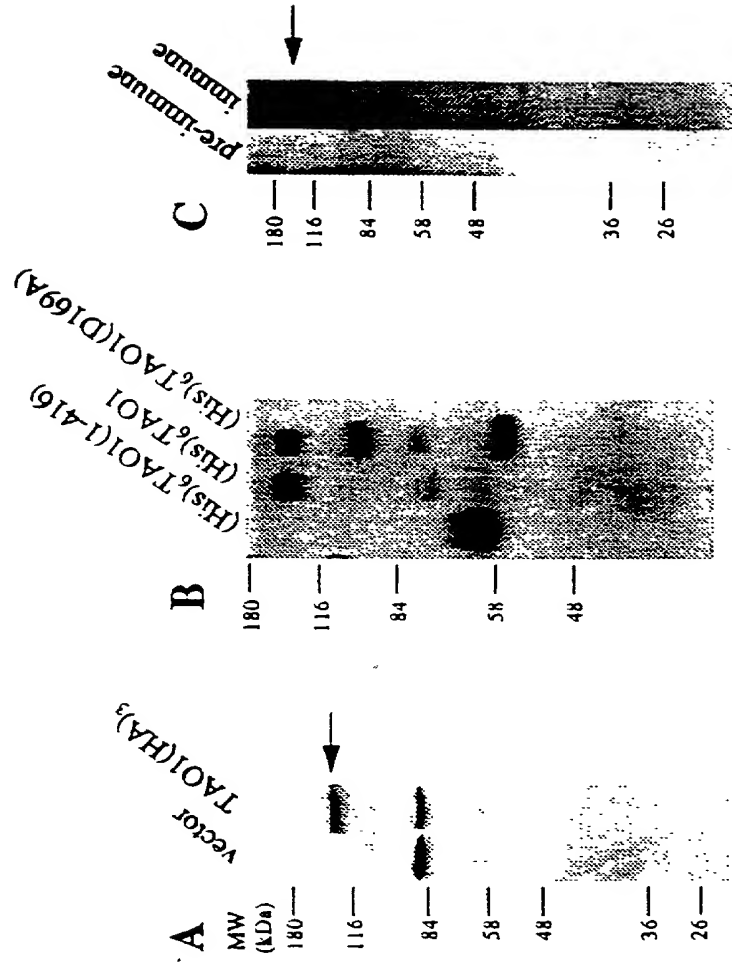




*FIGS. 3A and 3B*



**FIGS. 4A and 4B**



FIGS. 5A-5C

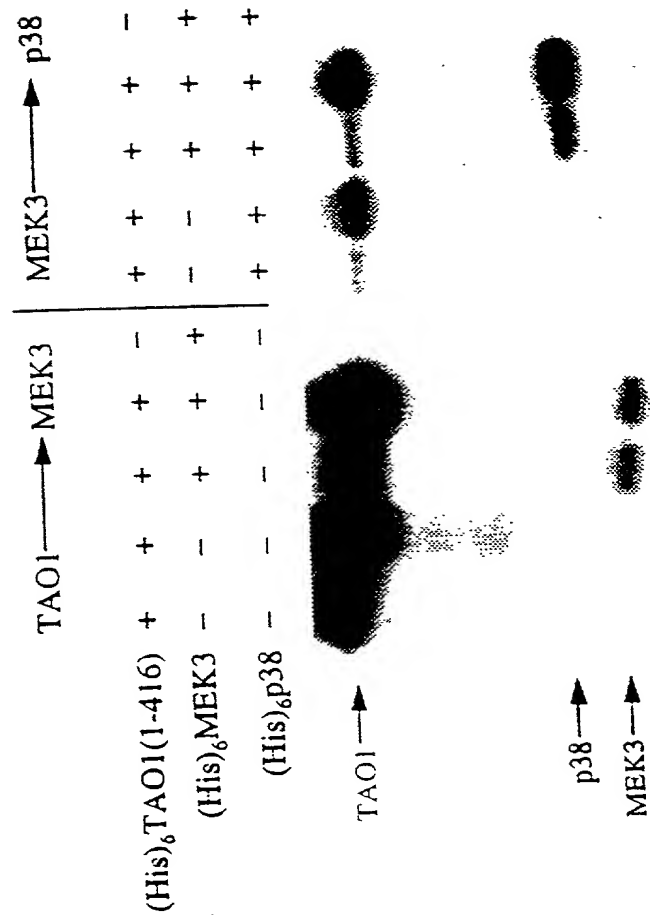


FIG. 6

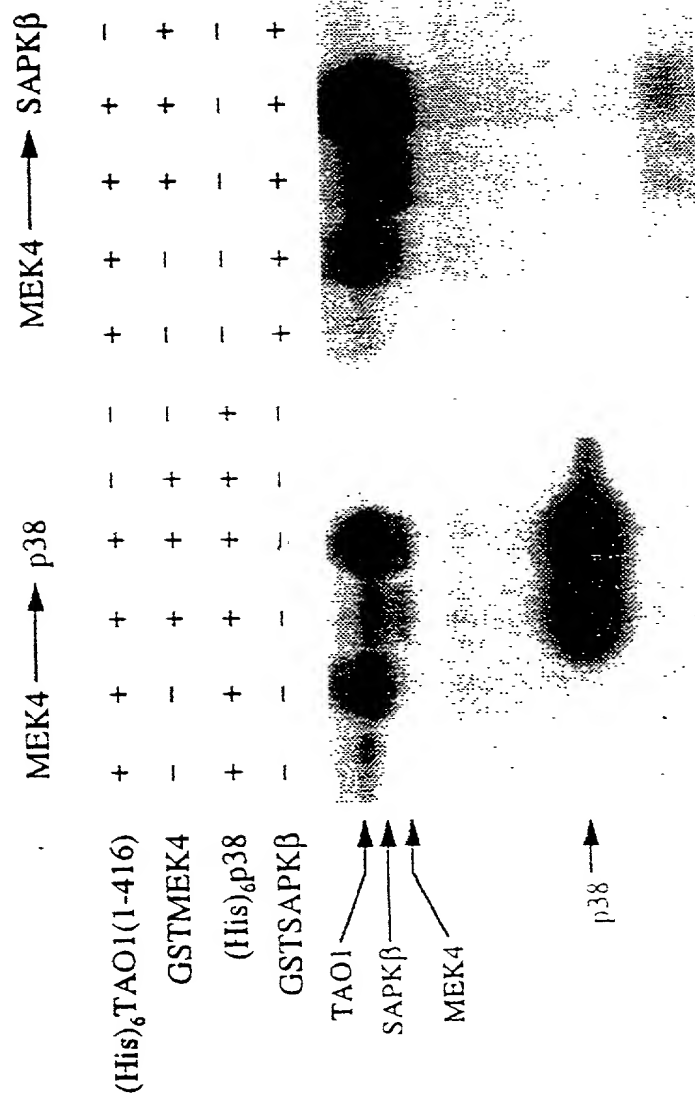


FIG. 7

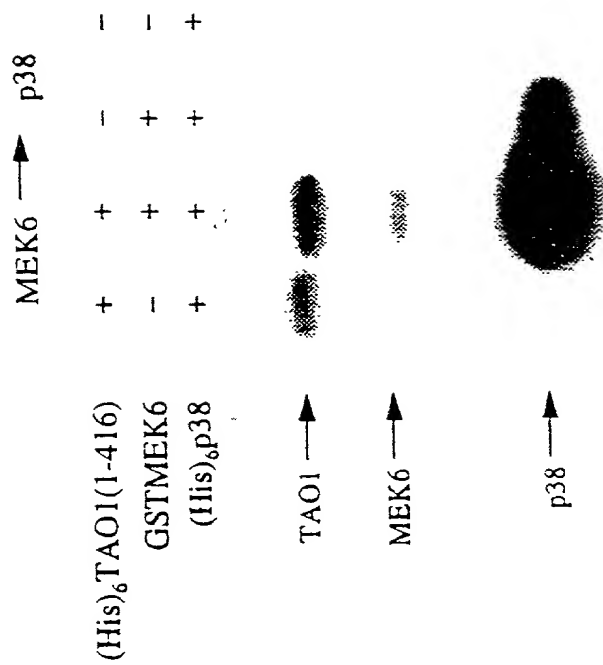
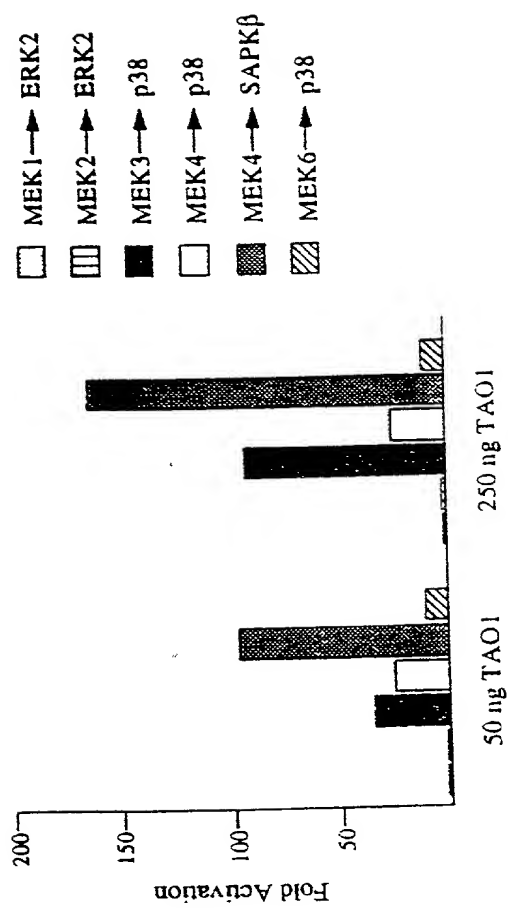
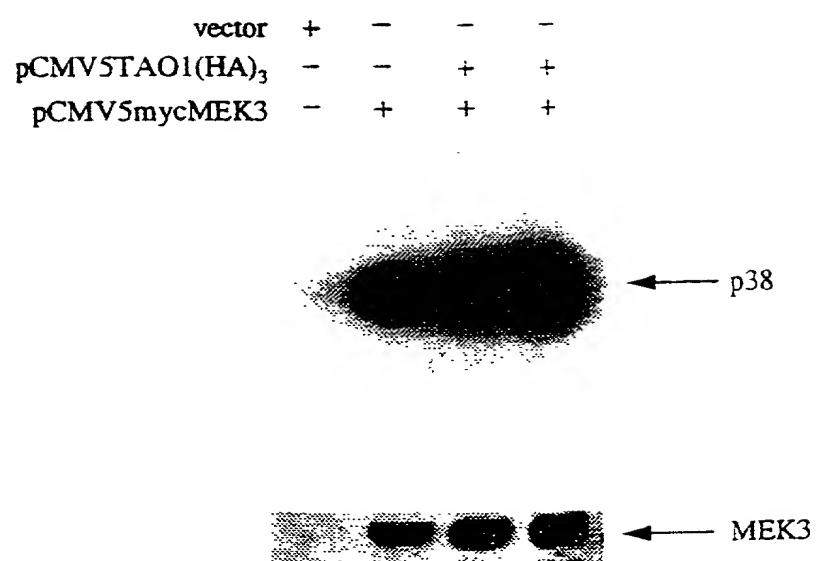


FIG. 8



**FIG. 9**



**FIG. 10**



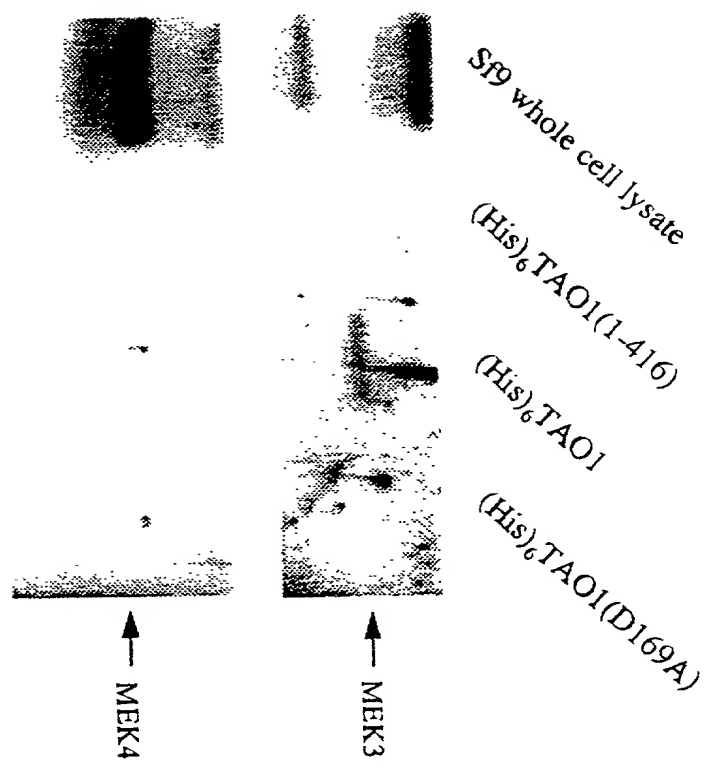


FIG. 11

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Sbjct: 12 ACCANTCACCAGTTGGAAGTTACTCCAAAGAATGAGCACAAAACAATCTTAAAGACACTG 71

Query: 2401 AAGGAGGAACAGACTCGGAAGTTAGCCATCTTGGCTGAGCAGTATGATCATAGCATTAAAT 2460
      | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Sbjct: 72 AAAGATGAGCAGACAAGAAAACCTTGCCATTTNGGCAGAGCAGTATGAACAGAGTATAAAT 131

Query: 2461 GAAATGCTCTCCACACAAGCTCTGCGTTTGGATGAAGCACAGGAAGCAGAATGCCAGGTT 2520
      | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Sbjct: 132 GAAATGATGGCCTCTCANGCGTTACGGCTAGATGAGGCTCAAGAAGCAGAATGCCAGGCC 191

Query: 2521 TTGAAGATGCAGCTACAGCAGGAACCTGGAGCTGTTGAATGCATATCAGAGCAAAATCAAG 2580
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Sbjct: 372 AGCGAGAGAATAAAGAACCTATTGGAAAGCAAGAGCGAGAGATTGGAAACTTT 425

```

**FIG. 12**





Variable	Mean	SD	Min	Max
Age	34.5	10.2	21	55
Gender	Male	1.0	0	1
Marital Status	Married	1.0	0	1
Education	High School	1.0	0	1
Occupation	Unemployed	1.0	0	1
Income	\$10,000	1.0	0	1
Health Status	Good	1.0	0	1
Smoking Status	Non-smoker	1.0	0	1
Alcohol Consumption	None	1.0	0	1
Exercise Frequency	None	1.0	0	1
Stress Level	Low	1.0	0	1
Sleep Quality	Good	1.0	0	1
Dietary Habits	Healthy	1.0	0	1
Family Size	2	1.0	1	4
Home Ownership	Owned	1.0	0	1
Vehicle Ownership	Owned	1.0	0	1
Insurance Status	Insured	1.0	0	1
Religious Beliefs	Religious	1.0	0	1
Political Views	Conservative	1.0	0	1
Travel Frequency	None	1.0	0	1
Pet Ownership	Owned	1.0	0	1
Golfing Frequency	None	1.0	0	1
Fishing Frequency	None	1.0	0	1
Gardening Frequency	None	1.0	0	1
Volunteering Frequency	None	1.0	0	1
Charitable Contributions	None	1.0	0	1
Philanthropic Activities	None	1.0	0	1
Community Involvement	None	1.0	0	1
Leadership Roles	None	1.0	0	1
Networking Frequency	None	1.0	0	1
Professional Development	None	1.0	0	1
Entrepreneurial Spirit	None	1.0	0	1
Investment Frequency	None	1.0	0	1
Risk Tolerance	Low	1.0	0	1
Financial Literacy	Low	1.0	0	1
Debt Management	Good	1.0	0	1
Asset Management	Good	1.0	0	1
Retirement Planning	Good	1.0	0	1
Estate Planning	Good	1.0	0	1
Legal Consultations	Good	1.0	0	1
Tax Planning	Good	1.0	0	1
Insurance Reviews	Good	1.0	0	1
Financial Goals	Clear	1.0	0	1
Investment Strategy	Conservative	1.0	0	1
Portfolio Diversification	Good	1.0	0	1
Market Research	Good	1.0	0	1
Financial Education	Good	1.0	0	1
Financial Literacy Score	75	10	60	90
Financial Literacy Score (Control)	75	10	60	90

Variable	Mean	SD	Min	Max
Age	34.5	10.2	21	55
Gender	Male	1.0	0	1
Marital Status	Married	1.0	0	1
Education	High School	1.0	0	1
Occupation	Unemployed	1.0	0	1
Income	\$10,000	1.0	0	1
Health Status	Good	1.0	0	1
Smoking Status	Non-smoker	1.0	0	1
Alcohol Consumption	None	1.0	0	1
Exercise Frequency	None	1.0	0	1
Stress Level	Low	1.0	0	1
Sleep Quality	Good	1.0	0	1
Dietary Habits	Healthy	1.0	0	1
Family Size	2	1.0	1	4
Home Ownership	Owned	1.0	0	1
Vehicle Ownership	Owned	1.0	0	1
Insurance Status	Insured	1.0	0	1
Religious Beliefs	Religious	1.0	0	1
Political Views	Conservative	1.0	0	1
Travel Frequency	None	1.0	0	1
Pet Ownership	Owned	1.0	0	1
Golfing Frequency	None	1.0	0	1
Fishing Frequency	None	1.0	0	1
Gardening Frequency	None	1.0	0	1
Volunteering Frequency	None	1.0	0	1
Charitable Contributions	None	1.0	0	1
Philanthropic Activities	None	1.0	0	1
Community Involvement	None	1.0	0	1
Leadership Roles	None	1.0	0	1
Networking Frequency	None	1.0	0	1
Professional Development	None	1.0	0	1
Entrepreneurial Spirit	None	1.0	0	1
Investment Frequency	None	1.0	0	1
Risk Tolerance	Low	1.0	0	1
Financial Literacy	Low	1.0	0	1
Debt Management	Good	1.0	0	1
Asset Management	Good	1.0	0	1
Retirement Planning	Good	1.0	0	1
Estate Planning	Good	1.0	0	1
Legal Consultations	Good	1.0	0	1
Tax Planning	Good	1.0	0	1
Insurance Reviews	Good	1.0	0	1
Financial Goals	Clear	1.0	0	1
Investment Strategy	Conservative	1.0	0	1
Portfolio Diversification	Good	1.0	0	1
Market Research	Good	1.0	0	1
Financial Education	Good	1.0	0	1
Financial Literacy Score	75	10	60	90
Financial Literacy Score (Control)	75	10	60	90

Variable	Mean	SD	Min	Max
Age	34.5	10.2	21	55
Gender	Male	1.0	0	1
Marital Status	Married	1.0	0	1
Education	High School	1.0	0	1
Occupation	Unemployed	1.0	0	1
Income	\$10,000	1.0	0	1
Health Status	Good	1.0	0	1
Smoking Status	Non-smoker	1.0	0	1
Alcohol Consumption	None	1.0	0	1
Exercise Frequency	None	1.0	0	1
Stress Level	Low	1.0	0	1
Sleep Quality	Good	1.0	0	1
Dietary Habits	Healthy	1.0	0	1
Family Size	2	1.0	1	4
Home Ownership	Owned	1.0	0	1
Vehicle Ownership	Owned	1.0	0	1
Insurance Status	Insured	1.0	0	1
Religious Beliefs	Religious	1.0	0	1
Political Views	Conservative	1.0	0	1
Travel Frequency	None	1.0	0	1
Pet Ownership	Owned	1.0	0	1
Golfing Frequency	None	1.0	0	1
Fishing Frequency	None	1.0	0	1
Gardening Frequency	None	1.0	0	1
Volunteering Frequency	None	1.0	0	1
Charitable Contributions	None	1.0	0	1
Philanthropic Activities	None	1.0	0	1
Community Involvement	None	1.0	0	1
Leadership Roles	None	1.0	0	1
Networking Frequency	None	1.0	0	1
Professional Development	None	1.0	0	1
Entrepreneurial Spirit	None	1.0	0	1
Investment Frequency	None	1.0	0	1
Risk Tolerance	Low	1.0	0	1
Financial Literacy	Low	1.0	0	1
Debt Management	Good	1.0	0	1
Asset Management	Good	1.0	0	1
Retirement Planning	Good	1.0	0	1
Estate Planning	Good	1.0	0	1
Legal Consultations	Good	1.0	0	1
Tax Planning	Good	1.0	0	1
Insurance Reviews	Good	1.0	0	1
Financial Goals	Clear	1.0	0	1
Investment Strategy	Conservative	1.0	0	1
Portfolio Diversification	Good	1.0	0	1
Market Research	Good	1.0	0	1
Financial Education	Good	1.0	0	1
Financial Literacy Score	75	10	60	90
Financial Literacy Score (Control)	75	10	60	90

Variable	Mean	SD	Min	Max
Age	34.5	10.2	18	65
Gender	Male	1.0	0	1
Marital Status	Married	1.0	0	1
Education	High School	1.0	0	1
Occupation	Unemployed	1.0	0	1
Income	\$10,000	1.0	0	1
Health Status	Good	1.0	0	1
Smoking Status	Non-smoker	1.0	0	1
Alcohol Consumption	None	1.0	0	1
Exercise Frequency	None	1.0	0	1
Stress Level	Low	1.0	0	1
Sleep Quality	Good	1.0	0	1
Dietary Habits	Healthy	1.0	0	1
Family Size	2	1.0	0	5
Home Ownership	Owned	1.0	0	1
Vehicle Ownership	Owned	1.0	0	1
Insurance Status	Insured	1.0	0	1
Religious Beliefs	Religious	1.0	0	1
Political Views	Conservative	1.0	0	1
Travel Frequency	None	1.0	0	1
Pet Ownership	Owned	1.0	0	1
Volunteering	Yes	1.0	0	1
Charitable Contributions	None	1.0	0	1
Community Involvement	Active	1.0	0	1
Life Satisfaction	High	1.0	0	1
Overall Well-being	Good	1.0	0	1

Variable	Mean	SD	Min	Max
Age	34.5	10.2	21	55
Gender	Male	1.0	0	1
Marital Status	Married	1.0	0	1
Education	High School	1.0	0	1
Occupation	Unemployed	1.0	0	1
Income	\$10,000	1.0	0	1
Health Status	Good	1.0	0	1
Smoking Status	Non-smoker	1.0	0	1
Alcohol Consumption	None	1.0	0	1
Exercise Frequency	None	1.0	0	1
Stress Level	Low	1.0	0	1
Sleep Quality	Good	1.0	0	1
Dietary Habits	Healthy	1.0	0	1
Family Size	2	1.0	1	4
Home Ownership	Owned	1.0	0	1
Vehicle Ownership	Owned	1.0	0	1
Insurance Status	Insured	1.0	0	1
Religious Beliefs	Religious	1.0	0	1
Political Views	Conservative	1.0	0	1
Travel Frequency	None	1.0	0	1
Pet Ownership	Owned	1.0	0	1
Golfing Frequency	None	1.0	0	1
Fishing Frequency	None	1.0	0	1
Gardening Frequency	None	1.0	0	1
Volunteering Frequency	None	1.0	0	1
Charitable Contributions	None	1.0	0	1
Philanthropic Activities	None	1.0	0	1
Community Involvement	None	1.0	0	1
Leadership Roles	None	1.0	0	1
Networking Frequency	None	1.0	0	1
Professional Development	None	1.0	0	1
Entrepreneurial Spirit	None	1.0	0	1
Investment Frequency	None	1.0	0	1
Risk Tolerance	Low	1.0	0	1
Financial Literacy	Low	1.0	0	1
Debt Management	Good	1.0	0	1
Asset Management	Good	1.0	0	1
Retirement Planning	Good	1.0	0	1
Estate Planning	Good	1.0	0	1
Legal Consultations	Good	1.0	0	1
Tax Planning	Good	1.0	0	1
Insurance Reviews	Good	1.0	0	1
Financial Goals	Clear	1.0	0	1
Investment Strategy	Conservative	1.0	0	1
Portfolio Diversification	Good	1.0	0	1
Market Research	Good	1.0	0	1
Financial Education	Good	1.0	0	1
Financial Literacy Score	75	10	60	90
Financial Literacy Score (Control)	75	10	60	90



*A*

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Sbjct: 91 GATGTCATAGTACTTGGCTGCCGGG 115

*B*

Query: 3200 CTCACCTGGGTACTACCGGGTGGAAGCTGTGCATATGGTATATTTT 3245  
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*FIG. 17A and 17B*





[illegible][illegible][illegible][illegible][illegible]



PATENT

Applicants	:	Melanie Cobb, Michele Hutchison, Zhu Chen and Kevin Berman
Filed	:	October 10, 2000
For	:	TAO PROTEIN KINASE POLYPEPTIDES AND METHODS OF USE THEREFOR

Date : October 10, 2000

## DECLARATION

I, Bonita R. Gladden, in accordance with 37 C.F.R. § 1.821(f) do hereby declare that, to the best of my knowledge, the content of the paper entitled "Sequence Listing" and the computer readable copy contained within the floppy disk are the same.

Dated this 10<sup>th</sup> day of October, 2000.

Bonita R. Gladden  
Legal Assistant

701 Fifth Avenue, Suite 6300  
Seattle, WA 98104-7092  
Phone: (206) 622-4900  
Fax: (206) 682-6031  
Wpn/860098/421C1Forms/Seq Dec

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      Hutchison, Michele
      Chen, Zhu
      Berman, Kevin
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atg cca tca act aac aga gca ggc agt cta aag gac cct gaa atc gca      168
Met Pro Ser Thr Asn Arg Ala Gly Ser Leu Lys Asp Pro Glu Ile Ala
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85 90 95

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Arg Glu His Thr Ala Trp Leu Val Met Glu Tyr Cys Leu Gly Ser Ala



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aat agt ctt cca gat gca tcg gat gac aag agt gag cta gac atg atg Asn Ser Leu Pro Asp Ala Ser Asp Asp Lys Ser Glu Leu Asp Met Met 370 375 380	1272
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aaa ctt att aag aaa cac caa gct tct atg gaa aaa gag gct aaa gtg Lys Leu Ile Lys Lys His Gln Ala Ser Met Glu Lys Glu Ala Lys Val 515 520 525	1704
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625	630	635	640
agg cag act cag aag gac tta gaa cat gca atg tta ctg cga cag cat	2088		
Arg Gln Thr Gln Lys Asp Leu Glu His Ala Met Leu Leu Arg Gln His			
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gaa tcc atg caa gaa ctg gag ttt cgc cac ctc aac act att cag aag	2136		
Glu Ser Met Gln Glu Leu Glu Phe Arg His Leu Asn Thr Ile Gln Lys			
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atg cgc tgt gag ttg atc aga ctg caa cat caa act gag ctt act aac	2184		
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675	680	685	
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Gln Leu Glu Tyr Asn Lys Arg Arg Glu Arg Glu Leu Arg Arg Lys His			
690	695	700	
gtc atg gaa gtt cga cag cag cct aag agt ttg aag tct aaa gaa ctc	2280		
Val Met Glu Val Arg Gln Gln Pro Lys Ser Leu Lys Ser Lys Glu Leu			
705	710	715	720
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Tyr Lys Ala Leu Arg Asn His Leu Leu Glu Thr Thr Pro Lys Ser Glu			
740	745	750	
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His Lys Ala Val Leu Lys Arg Leu Lys Glu Glu Gln Thr Arg Lys Leu			
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Ala Ile Leu Ala Glu Gln Tyr Asp His Ser Ile Asn Glu Met Leu Ser			
770	775	780	
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Thr Gln Ala Leu Arg Leu Asp Glu Ala Gln Glu Ala Glu Cys Gln Val			
785	790	795	800

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 Ser Lys Ile Lys Met Gln Ala Glu Ala Gln His Asp Arg Glu Leu Arg  
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 Glu Leu Glu Gln Arg Val Ser Leu Arg Arg Ala Leu Leu Glu Gln Lys  
 835 840 845

att gaa gaa gag atg ttg gct ttg cag aat gaa cgc aca gaa cga ata 2712  
 Ile Glu Glu Glu Met Leu Ala Leu Gln Asn Glu Arg Thr Glu Arg Ile  
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 Thr Pro Gln Ala Trp Gly His Pro Met Gln Gly Gly Pro Gln Pro Trp  
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 Gly Val Arg Asn Ser Pro Gln Ala Leu Arg Arg Thr Ala Ser Gly Gly  
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Asp Val Ala	Glu Leu	Phe Phe	Lys Asp	Asp Pro	Glu Lys	Leu Phe	Ser								
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Ser Gly Lys	Gln Ser	Asn Glu	Lys Trp	Gln Asp	Ile Ile	Lys Glu	Val								
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Arg Phe Leu	Gln Lys	Leu Arg	His Pro	Asn Thr	Ile Gln	Tyr Arg	Gly								
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tgt tac ctg	agg gag	cac aca	gct tgg	ctg gtg	atg gag	tat tgc	ctg								519
Cys Tyr Leu	Arg Glu	His Thr	Ala Trp	Leu Val	Met Glu	Tyr Cys	Leu								
		95		100			105								
ggg tca gct	tct gat	ctt ctc	gaa gtg	cac aag	aag ccg	ctg cag	gag								567
Gly Ser Ala	Ser Asp	Leu Leu	Glu Val	His Lys	Lys Pro	Leu Gln	Glu								







cag agg att ctg ggg gaa gaa atg gga acc ttt agt tcc agc cca caa	2679
Gln Arg Ile Leu Gly Glu Glu Met Gly Thr Phe Ser Ser Ser Pro Gln	
815 820 825	
aaa cat agg agt ctg gtt aat gag gaa gat tgg gat ata tct aaa gaa	2727
Lys His Arg Ser Leu Val Asn Glu Glu Asp Trp Asp Ile Ser Lys Glu	
830 835 840 845	
atg aag gag agt aga gtc cca tcc ctg gca tcc cag gag aga aat att	2775
Met Lys Glu Ser Arg Val Pro Ser Leu Ala Ser Gln Glu Arg Asn Ile	
850 855 860	
att ggc cag gaa gag gct ggg gca tgg aat ctg tgg gag aag gag cat	2823
Ile Gly Gln Glu Glu Ala Gly Ala Trp Asn Leu Trp Glu Lys Glu His	
865 870 875	
gga aac ctt gtg gat atg gag ttc aag ctt ggc tgg gtc cag ggt cca	2871
Gly Asn Leu Val Asp Met Glu Phe Lys Leu Gly Trp Val Gln Gly Pro	
880 885 890	
gtt ctg act cca gtg cct gag gag gaa gag gag gag gaa gag gag gga	2919
Val Leu Thr Pro Val Pro Glu Glu Glu Glu Glu Glu Glu Glu Glu Gly	
895 900 905	
ggg gct cca att gga acc ccc agg gac cct gga gat ggc tgt cct tcc	2967
Gly Ala Pro Ile Gly Thr Pro Arg Asp Pro Gly Asp Gly Cys Pro Ser	
910 915 920 925	
cca gat atc ccc cca gag cca cct cca tca cat ctg aga cag tac cct	3015
Pro Asp Ile Pro Pro Glu Pro Pro Pro Ser His Leu Arg Gln Tyr Pro	
930 935 940	
gct agc cag ctt cct gga ttc ttg tct cat ggc ctc ctg act ggc ctc	3063
Ala Ser Gln Leu Pro Gly Phe Leu Ser His Gly Leu Leu Thr Gly Leu	
945 950 955	
tcc ttt gca gtg ggg tcc tcc tct ggc ctc ttg ccc cta cta ctt ctg	3111
Ser Phe Ala Val Gly Ser Ser Ser Gly Leu Leu Pro Leu Leu Leu Leu	
960 965 970	
ctg cta ctc cca ttg ctg gca gcc cag ggt gga ggt ggc ttg cag gca	3159
Leu Leu Leu Pro Leu Leu Ala Ala Gln Gly Gly Gly Leu Gln Ala	
975 980 985	
gca ctg ctg gcc ctt gag gta gga cta gtg ggc ctg ggg gcc tcc tac	3207
Ala Leu Leu Ala Leu Glu Val Gly Leu Val Gly Leu Gly Ala Ser Tyr	
990 995 1000 1005	
ctg ttc ctt tgt aca gct cta cac ctg cca ccc agt ctg ttc tta ctc	3255
Leu Phe Leu Cys Thr Ala Leu His Leu Pro Pro Ser Leu Phe Leu Leu	
1010 1015 1020	
ctg gct cag ggc act gca ctg ggg gct gtc ctt agc ctg agc tgg cgc	3303
Leu Ala Gln Gly Thr Ala Leu Gly Ala Val Leu Ser Leu Ser Trp Arg	
1025 1030 1035	
aga ggc ctt atg ggt gtg cct ctg ggc ctt ggg gct gcc tgg ctc cta	3351
Arg Gly Leu Met Gly Val Pro Leu Gly Leu Gly Ala Ala Trp Leu Leu	









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<210> 5
<211> 414
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
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<223> N = a, c, t, g

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aaagatgagc agacaagaaa acttgccatt tnggcagagc agtatgaaca gagtataaat      120
gaaatgatgg cctctcangc gttacggcta gatgaggctc aagaagcaga atgccaggcc      180
ttgaggctac agctccagca ggaaatggag ctgctcaacg cctaccagag caaaatcaag      240
atgcaaacag aggcacaaca tgaacgtgag ctccagaagc tagagcagag agtgtctctg      300
cgcagagcac accttgagca gaagattgaa gaggagctgg ctgcccttca gaaggaacgc      360
agcgagagaa taaagaacct attggaaagg caagagcgag agattggaaa cttt          414

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<210> 6
<211> 314
<212> DNA
<213> Homo sapien

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<400> 6
gaacaaagtc atgccttaat agttctgctg atgttggcct ttcttgaggt attttctgca      60
agcagtaatc aacaaatctc cttaaaggag ctgtccattc attagactgt aacgttgggg      120
agtcattctg ggcaatgtga tataaggcac tcattgcatt catgttgaaa aggggcggct      180
tccgttccgc caattcaata caagtgatgc caagtgaacca aatatcaact ttcccatcat      240
actgtccttc atccatagct aagatcacct ctggagccat ccagtaagggt gtgcccacga      300
aggagttggc cagg          314

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<210> 7
<211> 370
<212> DNA
<213> Homo sapien

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<220>
<221> misc_feature
<222> (1)...(370)
<223> N = a, c, t, g

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<400> 7
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tgcttttcca ataggttctt tattctctcg ctgcgttcct tctgaagggc agccagctcc      120
tcttcaatct tctgctcaag gtgtgggtctg cgcagagaca ctctctgctc tagcttctgg      180
agctcacggt catgttggtg ctctgttngn atcttgattt ggntctggta ggcgttgagc      240
agctccattt cctgctggag ctgtagcctc aaggcctggc attctgcttc ttgagcctca      300
tctagccgta acgcttgaga ggccatcatt tcattttatac tctgttcata ctgctctgcc      360
aaaatggcaa          370

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<211> 190
<212> DNA
<213> Homo sapien

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tgcaaagtac agaccaaaaca gtataaagca ctcaagaatc accagttgga agttactcca      120
aagaatgagc acaaaaacaat cttaaagaca ctgaaagatg agcagacaag aaaacttgcc      180
atthttggcag          190

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<210> 9
<211> 65
<212> DNA
<213> Homo sapien

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 <212> DNA  
 <213> Homo sapien

<400> 15  
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 tacaagggga tcgacaacca caccaaggaa gtgggtggcca tcaagatcat 110

<210> 16  
 <211> 134  
 <212> DNA  
 <213> Homo sapien

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 cagatatcaa cttt 134

<210> 17  
 <211> 278  
 <212> PRT  
 <213> Saccharomyces cerevisiae

<400> 17  
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 Ile Ala Ala Leu Phe Ser Asn Lys Asp Pro Glu Gln Asp Leu Arg Glu  
 20 25 30  
 Ile Gly His Gly Ser Phe Gly Ala Val Tyr Phe Ala Tyr Asp Lys Lys  
 35 40 45  
 Asn Glu Gln Thr Val Ala Ile Lys Lys Met Asn Phe Ser Gly Lys Gln  
 50 55 60  
 Ala Val Glu Lys Trp Asn Asp Ile Leu Lys Glu Val Ser Phe Leu Asn  
 65 70 75 80  
 Thr Val Val His Pro His Ile Val Asp Tyr Lys Ala Cys Phe Leu Lys  
 85 90 95  
 Asp Thr Thr Cys Trp Leu Val Met Glu Tyr Cys Ile Gly Ser Ala Ala  
 100 105 110  
 Asp Ile Val Asp Val Leu Arg Lys Gly Met Arg Glu Val Glu Ile Ala  
 115 120 125  
 Ala Ile Cys Ser Gln Thr Leu Asp Ala Leu Arg Tyr Leu His Ser Leu  
 130 135 140  
 Lys Arg Ile His Arg Asp Ile Lys Ala Gly Asn Ile Leu Leu Ser Asp  
 145 150 155 160  
 His Ala Ile Val Lys Leu Ala Asp Phe Gly Ser Ala Ser Leu Val Asp  
 165 170 175  
 Pro Ala Gln Thr Phe Ile Gly Thr Pro Phe Phe Met Ala Pro Glu Val  
 180 185 190  
 Ile Leu Ala Met Asp Glu Gly His Tyr Thr Asp Arg Ala Asp Ile Trp  
 195 200 205  
 Ser Leu Gly Ile Thr Cys Ile Glu Leu Ala Glu Arg Arg Pro Pro Leu  
 210 215 220  
 Phe Ser Met Asn Ala Met Ser Ala Leu Tyr His Ile Ala Gln Asn Asp  
 225 230 235 240  
 Pro Pro Thr Leu Ser Pro Ile Asp Thr Ser Glu Gln Pro Glu Trp Ser  
 245 250 255  
 Leu Glu Phe Val Gln Phe Ile Asp Lys Cys Leu Arg Lys Pro Ala Glu

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20

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 <211> 19  
 <212> PRT  
 <213> Rattus norvegicus

<400> 24  
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 Leu Arg Lys

<210> 25  
 <211> 20  
 <212> PRT  
 <213> Rattus norvegicus

<400> 25  
 Arg Glu Leu Arg Glu Leu Glu Gln Arg Val Ser Leu Arg Arg Ala Leu  
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 Leu Glu Gln Lys  
                   20

<210> 26  
 <211> 8  
 <212> PRT  
 <213> Rattus norvegicus

<400> 26  
 His Arg Asp Ile Lys Ala Gly Asn  
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<210> 27  
 <211> 3781  
 <212> DNA  
 <213> C. elegans

<220>  
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 <222> (279)...(3227)

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 aaggaatcct ctggctaattg tgetctctcc gatcgacgac ggcgacgcc tctctgattt 120  
 ttactcatcc atcaacaaca aaatcaaaca agacaagaaa aacatcagaa aatttcaaaa 180  
 attaataaca atacacattt attaataatc aaaaattcat ttctggtggc gccgcgcttc 240  
 tcgaatatac ggagaacgga ggaggtggtg gagttacg atg gcg cct gcc gtc tta 296  
   Met Ala Pro Ala Val Leu  
   1                  5

caa aaa ccc ggt gtt atc aag gat cca tcg att gct gca ttg ttc agt 344  
 Gln Lys Pro Gly Val Ile Lys Asp Pro Ser Ile Ala Ala Leu Phe Ser  
                   10                  15                  20

aat aag gat cca gag cag aga tat caa gat tta aga gaa att gga cat 392  
 Asn Lys Asp Pro Glu Gln Arg Tyr Gln Asp Leu Arg Glu Ile Gly His  
                   25                  30                  35



Val	Gln	Phe	Ile	Asp	Lys	Cys	Leu	Arg	Lys	Pro	Ala	Glu	Glu	Arg	Met	
		265					270					275				
tca	gct	gaa	gaa	tgc	ttt	cga	cat	cca	ttc	att	caa	cgg	tct	cgc	cca	1160
Ser	Ala	Glu	Glu	Cys	Phe	Arg	His	Pro	Phe	Ile	Gln	Arg	Ser	Arg	Pro	
		280				285					290					
tca	gac	aca	att	cag	gaa	ctc	att	cag	aga	acg	aaa	aat	atg	gta	tta	1208
Ser	Asp	Thr	Ile	Gln	Glu	Leu	Ile	Gln	Arg	Thr	Lys	Asn	Met	Val	Leu	
295					300					305					310	
gag	ttg	gat	aat	ttt	caa	tac	aaa	aag	atg	aga	aaa	ctc	atg	tat	ttg	1256
Glu	Leu	Asp	Asn	Phe	Gln	Tyr	Lys	Lys	Met	Arg	Lys	Leu	Met	Tyr	Leu	
				315					320					325		
gat	gaa	aca	gaa	gga	aaa	gaa	gga	agt	gaa	gga	aat	gga	gca	tct	gat	1304
Asp	Glu	Thr	Glu	Gly	Lys	Glu	Gly	Ser	Glu	Gly	Asn	Gly	Ala	Ser	Asp	
			330					335					340			
gat	tta	gat	ttt	cat	gga	aat	gaa	gct	aat	tca	att	gga	aga	gca	gga	1352
Asp	Leu	Asp	Phe	His	Gly	Asn	Glu	Ala	Asn	Ser	Ile	Gly	Arg	Ala	Gly	
		345					350					355				
gat	tct	gcg	tca	tct	cga	agt	gct	tct	ctt	act	tct	ttc	cga	tca	atg	1400
Asp	Ser	Ala	Ser	Ser	Arg	Ser	Ala	Ser	Leu	Thr	Ser	Phe	Arg	Ser	Met	
	360					365				370						
cag	agt	agt	gga	gga	gct	ggt	ctt	tta	gtg	tcc	acc	aat	acg	acg	ggt	1448
Gln	Ser	Ser	Gly	Gly	Ala	Gly	Leu	Leu	Val	Ser	Thr	Asn	Thr	Thr	Gly	
375					380					385					390	
gct	atg	gat	aat	gtg	cat	gga	tcc	tct	gga	tac	ggt	aat	gga	agt	agt	1496
Ala	Met	Asp	Asn	Val	His	Gly	Ser	Ser	Gly	Tyr	Gly	Asn	Gly	Ser	Ser	
				395					400					405		
tcg	acg	acg	agc	tcc	gca	cgc	cgc	cgt	cct	cca	att	cct	tcg	caa	atg	1544
Ser	Thr	Thr	Ser	Ser	Ala	Arg	Arg	Arg	Pro	Pro	Ile	Pro	Ser	Gln	Met	
			410					415					420			
ctc	tct	tct	aca	tca	acg	tct	ggt	gtt	gga	act	atg	ccg	agt	cat	gga	1592
Leu	Ser	Ser	Thr	Ser	Thr	Ser	Gly	Val	Gly	Thr	Met	Pro	Ser	His	Gly	
		425					430					435				
tca	gtt	gga	gca	tcg	att	acg	gcg	atc	gca	gtc	aat	cca	aca	ccg	tct	1640
Ser	Val	Gly	Ala	Ser	Ile	Thr	Ala	Ile	Ala	Val	Asn	Pro	Thr	Pro	Ser	
	440					445					450					
cct	tca	gaa	cct	atc	cca	aca	tca	caa	cca	aca	tcg	aaa	tca	gaa	tca	1688
Pro	Ser	Glu	Pro	Ile	Pro	Thr	Ser	Gln	Pro	Thr	Ser	Lys	Ser	Glu	Ser	
455					460					465					470	
tct	tct	ata	ctc	gaa	act	gca	cac									

cga ata gcc acg ttg caa aat cac aaa ttc gcg acg ctt cgt tcc cag	1832
Arg Ile Ala Thr Leu Gln Asn His Lys Phe Ala Thr Leu Arg Ser Gln	
505 510 515	
aga ata atc aat cag gaa caa gaa gaa tat acg aaa gag aac aat atg	1880
Arg Ile Ile Asn Gln Glu Gln Glu Glu Tyr Thr Lys Glu Asn Asn Met	
520 525 530	
tat gag caa atg agc aag tac aag cat cta cga caa gca cat cac aaa	1928
Tyr Glu Gln Met Ser Lys Tyr Lys His Leu Arg Gln Ala His His Lys	
535 540 545 550	
gag ctc caa caa ttt gaa gaa cga tgt gca tta gat aga gag caa ctg	1976
Glu Leu Gln Gln Phe Glu Glu Arg Cys Ala Leu Asp Arg Glu Gln Leu	
555 560 565	
cgt gtg aaa atg gat cga gaa ctc gaa caa ttg aca acg aca tac tcg	2024
Arg Val Lys Met Asp Arg Glu Leu Glu Gln Leu Thr Thr Thr Tyr Ser	
570 575 580	
aaa gaa aag atg aga gtg agg tgt tca cag aat aat gaa cta gac aaa	2072
Lys Glu Lys Met Arg Val Arg Cys Ser Gln Asn Asn Glu Leu Asp Lys	
585 590 595	
cgg aaa aaa gat atc gaa gat ggg gag aaa aag atg aaa aag acg aaa	2120
Arg Lys Lys Asp Ile Glu Asp Gly Glu Lys Lys Met Lys Lys Thr Lys	
600 605 610	
aat agt caa aat cag cag cag atg aaa ctg tat tca gcg caa caa ttg	2168
Asn Ser Gln Asn Gln Gln Gln Met Lys Leu Tyr Ser Ala Gln Gln Leu	
615 620 625 630	
aaa gaa tac aag tat aac aag gag gca cag aaa aca cga tta cga agt	2216
Lys Glu Tyr Lys Tyr Asn Lys Glu Ala Gln Lys Thr Arg Leu Arg Ser	
635 640 645	
ctg aac atg cct cga agt act tat gag aac gca atg aaa gaa gtg aaa	2264
Leu Asn Met Pro Arg Ser Thr Tyr Glu Asn Ala Met Lys Glu Val Lys	
650 655 660	
gcc gat ctg aat cga gtg aaa gat gca cgg gaa aat gat ttt gac gag	2312
Ala Asp Leu Asn Arg Val Lys Asp Ala Arg Glu Asn Asp Phe Asp Glu	
665 670 675	
aag ctt cgt gca gaa ctt gaa gat gaa att gta agg tat cgc agg caa	2360
Lys Leu Arg Ala Glu Leu Glu Asp Glu Ile Val Arg Tyr Arg Arg Gln	
680 685 690	
caa ctc agt aat ctt cat caa ttg gaa gaa caa ttg gat gat gaa gac	2408
Gln Leu Ser Asn Leu His Gln Leu Glu Glu Gln Leu Asp Asp Glu Asp	
695 700 705 710	
gta aac gtg caa gaa cgc caa atg gac acg cgt cac gga tta ctg tca	2456
Val Asn Val Gln Glu Arg Gln Met Asp Thr Arg His Gly Leu Leu Ser	
715 720 725	
aag cag cat gaa atg acg cgc gat ttg gaa ata cag cat ctc aac gag	2504

Lys	Gln	His	Glu	Met	Thr	Arg	Asp	Leu	Glu	Ile	Gln	His	Leu	Asn	Glu	
			730					735					740			
ctt	cac	gcg	atg	aaa	aaa	cga	cat	ttg	gag	aca	caa	cac	gag	gcg	gaa	2552
Leu	His	Ala	Met	Lys	Lys	Arg	His	Leu	Glu	Thr	Gln	His	Glu	Ala	Glu	
			745				750					755				
tcg	gca	agt	caa	aat	gag	tac	aca	cag	agg	caa	cag	gat	gaa	ttg	aga	2600
Ser	Ala	Ser	Gln	Asn	Glu	Tyr	Thr	Gln	Arg	Gln	Gln	Asp	Glu	Leu	Arg	
			760				765					770				
aaa	aag	cat	gcg	atg	cag	tca	aga	caa	cag	cca	aga	gat	tta	aag	atc	2648
Lys	Lys	His	Ala	Met	Gln	Ser	Arg	Gln	Gln	Pro	Arg	Asp	Leu	Lys	Ile	
			775				780				785				790	
caa	gaa	gca	caa	att	cga	aaa	caa	tac	cga	caa	gtt	gtg	aag	act	cag	2696
Gln	Glu	Ala	Gln	Ile	Arg	Lys	Gln	Tyr	Arg	Gln	Val	Val	Lys	Thr	Gln	
			795				800					805				
act	cgc	caa	ttt	aag	ctc	tac	ctt	aca	caa	atg	gtg	caa	gta	gtt	cca	2744
Thr	Arg	Gln	Phe	Lys	Leu	Tyr	Leu	Thr	Gln	Met	Val	Gln	Val	Val	Pro	
			810				815					820				
aaa	gat	gaa	caa	aaa	gag	ctc	acg	tct	cga	cta	aaa	cag	gat	caa	atg	2792
Lys	Asp	Glu	Gln	Lys	Glu	Leu	Thr	Ser	Arg	Leu	Lys	Gln	Asp	Gln	Met	
			825				830					835				
caa	aaa	gtc	gca	ctt	ctt	gct	tca	caa	tac	gaa	agt	caa	atc	aaa	aaa	2840
Gln	Lys	Val	Ala	Leu	Leu	Ala	Ser	Gln	Tyr	Glu	Ser	Gln	Ile	Lys	Lys	
			840				845					850				
atg	gtt	cag	gat	aag	aca	gtg	aag	ctc	gag	tcg	tgg	caa	gaa	gat	gaa	2888
Met	Val	Gln	Asp	Lys	Thr	Val	Lys	Leu	Glu	Ser	Trp	Gln	Glu	Asp	Glu	
			855				860				865				870	
caa	cgg	gtt	ctt	agt	gag	aag	ttg	gag	aaa	gaa	ttg	gaa	gaa	ttg	att	2936
Gln	Arg	Val	Leu	Ser	Glu	Lys	Leu	Glu	Lys	Glu	Leu	Glu	Glu	Leu	Ile	
			875				880					885				
gct	tat	cag	aag	aag	acg	aga	gcc	aca	tta	gaa	gag	cag	att	aaa	aag	2984
Ala	Tyr	Gln	Lys	Lys	Thr	Arg	Ala	Thr	Leu	Glu	Glu	Gln	Ile	Lys	Lys	
			890				895					900				
gaa	cgt	acg	gca	ctc	gaa	gaa	cga	att	ggc	aca	cga	cgt	gca	atg	ctt	3032
Glu	Arg	Thr	Ala	Leu	Glu	Glu	Arg	Ile	Gly	Thr	Arg	Arg	Ala	Met	Leu	
			905				910					915				
gaa	cag	aag	att	att	gaa	gaa	cgc	gaa	caa	atg	gga	gaa	atg	cgt	cga	3080
Glu	Gln	Lys	Ile	Ile	Glu	Glu	Arg	Glu	Gln	Met	Gly	Glu	Met	Arg	Arg	
			920				925				930					
cta	aag	aag	gag	caa	atc	cgt	gat	cga	cac	agt	caa	gaa	cgc	cat	cgt	3128
Leu	Lys	Lys	Glu	Gln	Ile	Arg	Asp	Arg	His	Ser	Gln	Glu	Arg	His	Arg	
			935				940				945				950	
ctc	gag	aat	cat	ttc	gta	cgg	acg	ggc	tcg	acg	agc	aga	agt	tct	ggc	3176
Leu	Glu	Asn	His	Phe	Val	Arg	Thr	Gly	Ser	Thr	Ser	Arg	Ser	Ser	Gly	

ggg atc gct cct ggt gtt ggg aat tca agc agt att cag atg gct atg 3224  
 Gly Ile Ala Pro Gly Val Gly Asn Ser Ser Ser Ile Gln Met Ala Met  
                   970                                  980

tag aatgatgttg tctcttaatt ctactacact tcgggtcgtc taccaattgt 3277  
 \*

tttctttttt tgtcgtttta tacaaaatgt atgcttgtat ggacaattaa ttgatagtat 3337  
 cttccaaata tcttccatct aattacggtt ccagttcgtc tttttctatg ttttttctgc 3397  
 accaccctca ttctctcact ttctccatgt cttctccctt ctttttttagc tcccaaaatc 3457  
 caattcttct gtgatagatg aaaaccacgg ctaaacccaa tggggatcgg tatcccgatc 3517  
 tccgggatac agtaaccoga aaatacgtgc aatacttctc aactataccc aatttttctg 3577  
 tcttcacctt ctcttctctaa ttctattcag aaacgaaact ttaatcgggtg ctctcaacgt 3637  
 tgttttctct attttctcta atatttatag tctatttgat atctgaacc catcatcata 3697  
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 taattattta aaaaaaaaaa aaaa 3781

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 <212> PRT  
 <213> C. elegans

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                   20                  25                  30  
 Leu Arg Glu Ile Gly His Gly Ser Phe Gly Ala Val Tyr Phe Ala Tyr  
                   35                  40                  45  
 Asp Lys Lys Asn Glu Gln Thr Val Ala Ile Lys Lys Met Asn Phe Ser  
                   50                  55                  60  
 Gly Lys Gln Ala Val Glu Lys Trp Asn Asp Ile Leu Lys Glu Val Ser  
   65                  70                  75                  80  
 Phe Leu Asn Thr Val Val His Pro His Ile Val Asp Tyr Lys Ala Cys  
                   85                  90                  95  
 Phe Leu Lys Asp Thr Thr Cys Trp Leu Val Met Glu Tyr Cys Ile Gly  
                   100                  105                  110  
 Ser Ala Ala Asp Ile Val Asp Val Leu Arg Lys Gly Met Arg Glu Val  
                   115                  120                  125  
 Glu Ile Ala Ala Ile Cys Ser Gln Thr Leu Asp Ala Leu Arg Tyr Leu  
                   130                  135                  140  
 His Ser Leu Lys Arg Ile His Arg Asp Ile Lys Ala Gly Asn Ile Leu  
   145                  150                  155                  160  
 Leu Ser Asp His Ala Ile Val Lys Leu Ala Asp Phe Gly Ser Ala Ser  
                   165                  170                  175  
 Leu Val Asp Pro Ala Gln Thr Phe Ile Gly Thr Pro Phe Phe Met Ala  
                   180                  185                  190  
 Pro Glu Val Ile Leu Ala Met Asp Glu Gly His Tyr Thr Asp Arg Ala  
                   195                  200                  205  
 Asp Ile Trp Ser Leu Gly Ile Thr Cys Ile Glu Leu Ala Glu Arg Arg  
   210                  215                  220  
 Pro Pro Leu Phe Ser Met Asn Ala Met Ser Ala Leu Tyr His Ile Ala  
   225                  230                  235                  240  
 Gln Asn Asp Pro Pro Thr Leu Ser Pro Ile Asp Thr Ser Glu Gln Pro  
                   245                  250                  255  
 Glu Trp Ser Leu Glu Phe Val Gln Phe Ile Asp Lys Cys Leu Arg Lys

			260						265						270				
Pro	Ala	Glu	Glu	Arg	Met	Ser	Ala	Glu	Glu	Cys	Phe	Arg	His	Pro	Phe				
		275					280					285							
Ile	Gln	Arg	Ser	Arg	Pro	Ser	Asp	Thr	Ile	Gln	Glu	Leu	Ile	Gln	Arg				
	290					295					300								
Thr	Lys	Asn	Met	Val	Leu	Glu	Leu	Asp	Asn	Phe	Gln	Tyr	Lys	Lys	Met				
305					310					315					320				
Arg	Lys	Leu	Met	Tyr	Leu	Asp	Glu	Thr	Glu	Gly	Lys	Glu	Gly	Ser	Glu				
				325					330					335					
Gly	Asn	Gly	Ala	Ser	Asp	Asp	Leu	Asp	Phe	His	Gly	Asn	Glu	Ala	Asn				
			340					345					350						
Ser	Ile	Gly	Arg	Ala	Gly	Asp	Ser	Ala	Ser	Ser	Arg	Ser	Ala	Ser	Leu				
		355					360					365							
Thr	Ser	Phe	Arg	Ser	Met	Gln	Ser	Ser	Gly	Gly	Ala	Gly	Leu	Leu	Val				
	370					375					380								
Ser	Thr	Asn	Thr	Thr	Gly	Ala	Met	Asp	Asn	Val	His	Gly	Ser	Ser	Gly				
385					390					395					400				
Tyr	Gly	Asn	Gly	Ser	Ser	Ser	Thr	Thr	Ser	Ser	Ala	Arg	Arg	Arg	Pro				
				405					410					415					
Pro	Ile	Pro	Ser	Gln	Met	Leu	Ser	Ser	Thr	Ser	Thr	Ser	Gly	Val	Gly				
			420					425					430						
Thr	Met	Pro	Ser	His	Gly	Ser	Val	Gly	Ala	Ser	Ile	Thr	Ala	Ile	Ala				
		435					440					445							
Val	Asn	Pro	Thr	Pro	Ser	Pro	Ser	Glu	Pro	Ile	Pro	Thr	Ser	Gln	Pro				
	450					455					460								
Thr	Ser	Lys	Ser	Glu	Ser	Ser	Ser	Ile	Leu	Glu	Thr	Ala	His	Asp	Asp				
465					470					475					480				
Pro	Leu	Asp	Thr	Ser	Ile	Arg	Ala	Pro	Val	Lys	Asp	Leu	His	Met	Pro				
				485					490					495					
His	Arg	Ala	Val	Lys	Glu	Arg	Ile	Ala	Thr	Leu	Gln	Asn	His	Lys	Phe				
			500					505					510						
Ala	Thr	Leu	Arg	Ser	Gln	Arg	Ile	Ile	Asn	Gln	Glu	Gln	Glu	Glu	Tyr				
		515					520					525							
Thr	Lys	Glu	Asn	Asn	Met	Tyr	Glu	Gln	Met	Ser	Lys	Tyr	Lys	His	Leu				
	530					535					540								
Arg	Gln	Ala	His	His	Lys	Glu	Leu	Gln	Gln	Phe	Glu	Glu	Arg	Cys	Ala				
545					550					555					560				
Leu	Asp	Arg	Glu	Gln	Leu	Arg	Val	Lys	Met	Asp	Arg	Glu	Leu	Glu	Gln				
				565					570					575					
Leu	Thr	Thr	Thr	Tyr	Ser	Lys	Glu	Lys	Met	Arg	Val	Arg	Cys	Ser	Gln				
			580					585					590						
Asn	Asn	Glu	Leu	Asp	Lys	Arg	Lys	Lys	Asp	Ile	Glu	Asp	Gly	Glu	Lys				
		595					600					605							

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              725              730              735
Ile Gln His Leu Asn Glu Leu His Ala Met Lys Lys Arg His Leu Glu
              740              745              750
Thr Gln His Glu Ala Glu Ser Ala Ser Gln Asn Glu Tyr Thr Gln Arg
              755              760              765
Gln Gln Asp Glu Leu Arg Lys Lys His Ala Met Gln Ser Arg Gln Gln
              770              775              780
Pro Arg Asp Leu Lys Ile Gln Glu Ala Gln Ile Arg Lys Gln Tyr Arg
785              790              795              800
Gln Val Val Lys Thr Gln Thr Arg Gln Phe Lys Leu Tyr Leu Thr Gln
              805              810              815
Met Val Gln Val Val Pro Lys Asp Glu Gln Lys Glu Leu Thr Ser Arg
              820              825              830
Leu Lys Gln Asp Gln Met Gln Lys Val Ala Leu Leu Ala Ser Gln Tyr
              835              840              845
Glu Ser Gln Ile Lys Lys Met Val Gln Asp Lys Thr Val Lys Leu Glu
              850              855              860
Ser Trp Gln Glu Asp Glu Gln Arg Val Leu Ser Glu Lys Leu Glu Lys
865              870              875              880
Glu Leu Glu Glu Leu Ile Ala Tyr Gln Lys Lys Thr Arg Ala Thr Leu
              885              890              895
Glu Glu Gln Ile Lys Lys Glu Arg Thr Ala Leu Glu Glu Arg Ile Gly
              900              905              910
Thr Arg Arg Ala Met Leu Glu Gln Lys Ile Ile Glu Glu Arg Glu Gln
              915              920              925
Met Gly Glu Met Arg Arg Leu Lys Lys Glu Gln Ile Arg Asp Arg His
              930              935              940
Ser Gln Glu Arg His Arg Leu Glu Asn His Phe Val Arg Thr Gly Ser
945              950              955              960
Thr Ser Arg Ser Ser Gly Gly Ile Ala Pro Gly Val Gly Asn Ser Ser
              965              970              975
Ser Ile Gln Met Ala Met
              980

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